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VOLUME 37

October, 1937-January, 1938 (inclusive)

NEW YORK

Press of
THOMAS J. GRIFFITHS SONS, INC.,
Utica, N. Y.

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Proceedings
of the
Society
for
Experimental Biology and Medicine

VOL. 37. OCTOBER, 1937. No. 1.

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NEW YORK	
New York Academy of Medicine	October 20, 1937
PEIPING	
Peiping Union Medical College	May 27, 1937

9430

Effect of Total Sympathectomy on Experimental Renal Hypertension in Dogs.*

LOUIS K. ALPERT, ALF S. ALVING AND K. S. GRIMSON.

From the Lasker Foundation for Medical Research, the Department of Medicine, and the Department of Surgery, University of Chicago.

Excision of the splanchnic nerves and the lower 4 dorsal sympathetic ganglia has been shown by Goldblatt, Gross and Hanzal[1] to have no effect on experimental renal hypertension. Grimson[2] has found that in normal dogs total sympathectomy produces a temporary fall in blood pressure.

The effect of total sympathectomy on experimental renal hyper-

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* This work was aided by a grant from the Douglas Smith Foundation. Reference was made to this work in a report presented by D. Phemister and K. Grimson at the meeting of the American Surgical Association in New York City, June 3, 1937 (*Surgery*, 1937, **2**, 472).

[1] Goldblatt, H., Gross, J., and Hanzal, R. F., *J. Exp. Med.*, 1937, **65**, 233.

[2] Grimson, K. S., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 235.

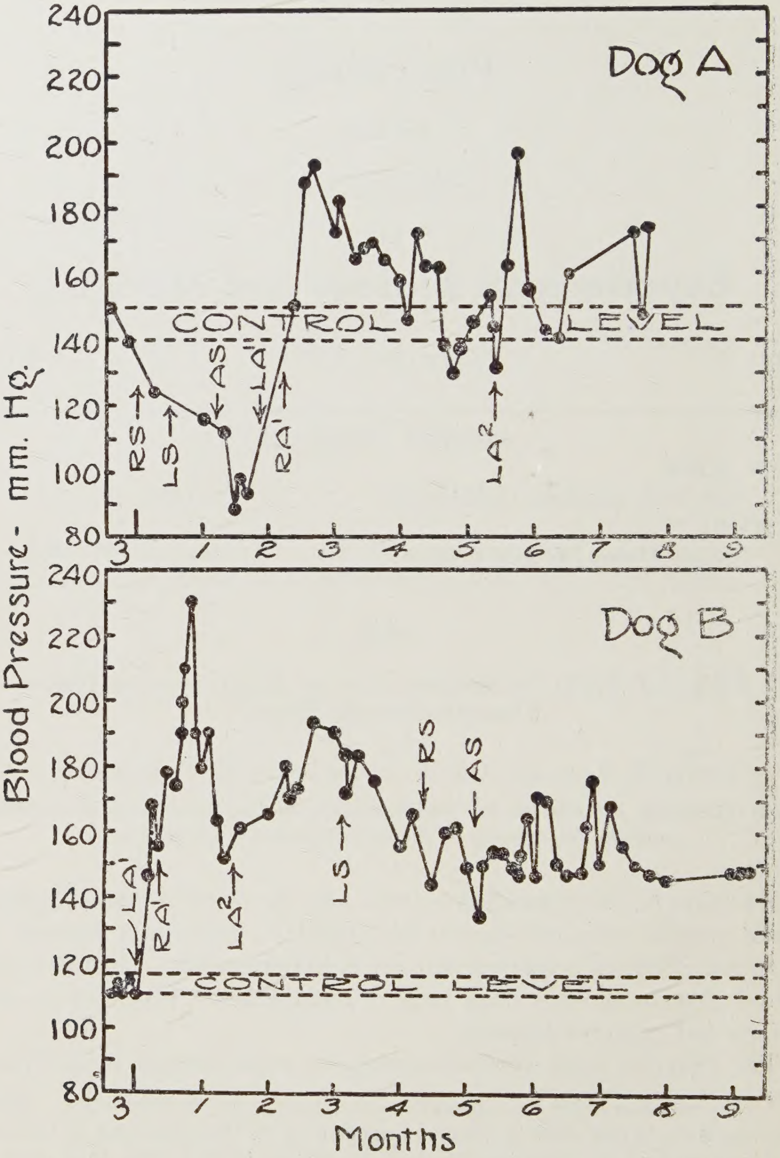

FIG. 1.

Effect of total sympathectomy on experimental renal hypertension. RS indicates right thoracic sympathectomy; LS, left thoracic sympathectomy; AS, abdominal sympathectomy; LA, clamp applied to left renal artery; RA, clamp applied to right renal artery.

tension as produced by Goldblatt, *et al.*,[3] has been studied in 2 dogs. Blood pressures throughout the study were obtained by direct arterial puncture, the lowest sustained readings during a 10-minute period being charted (Fig. 1). In each dog control pressures were followed for 3 months before any operative procedure was performed. In Dog A the control readings ranged from 140 to 150 mm. Hg., and dropped to 88 mm. following total sympathectomy. After application of Goldblatt clamps to both renal arteries the blood pressure rose to 193 mm. After a gradual return of the blood pressure to the control level, the left clamp was further tightened, and the pressure again rose to 198 mm. A subsequent return to near the control level again occurred. Freeman and Page[4] have also shown that total sympathectomy does not interfere with the development of hypertension due to bilateral renal ischemia.

In Dog B, whose control blood pressure was 110 to 116 mm., a rise to 230 mm. occurred following bilateral application of clamps to the renal arteries. When a tendency toward a lower level appeared, the left clamp was tightened, with a return of the blood pressure to 190 mm. Following total sympathectomy the blood pressure dropped to a lower level but after 4 months was still maintained at 30 mm. above the control level. Observations have not been continued for a sufficient length of time to determine whether the drop in blood pressure after sympathectomy is transient, as is the case in non-hypertensive dogs.[2]

Summary. In one dog with sustained hypertension established by application of Goldblatt clamps to both renal arteries, total sympathectomy produced a moderate drop in the blood pressure, which, however, has remained well above the control level. In another dog, previously sympathectomized, hypertension could be produced by bilateral application of Goldblatt clamps.

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[3] Goldblatt, H., Lynch, J., Hanzal, R. F., and Summerville, W. W., *J. Exp. Med.*, 1934, **59**, 347.

[4] Freeman, N. E., and Page, I. H., Report of Meetings of American Heart Association, Atlantic City, June 7 and 8, 1937. *Surgery*, 1937, **2**, 487.

9431

Effect of Dinitrophenol on Experimental Diabetes.

A. Hughes Bryan, Henry T. Ricketts and William C. Dine.
(Introduced by Louis Leiter.)

From the Department of Medicine, The University of Chicago.

The metabolic action of 2-4-dinitrophenol has been the subject of several recent and informative studies. Thus the greatly increased oxygen consumption characteristic of its administration to animals and man seems from the observations of Hall, Tainter, Cutting and coworkers[1, 2] to be largely at the expense of fat as a fuel, at least when the stimulus augmenting metabolism is not too abrupt and overwhelming. There is general agreement that dinitrophenol does not increase the output of nitrogen in the urine[1-5] and hence protein catabolism seems to be little affected. The injection of large and generally fatal doses into animals causes some elevation of the blood sugar and a considerable loss of liver and muscle glycogen, but smaller doses have little effect on the blood sugar.[2, 6, 7] The glycemic effect of large doses of dinitrophenol is not dependent upon asphyxia[8] and examination of the blood shows no very significant shift in the acid-base balance.[2, 8, 9] Studies of the ammonia, organic acid and ketone body excretion of patients administered dinitrophenol indicate that the increased oxidation of fat is complete.[1, 3, 4] In fact, as Tainter, Cutting, and Hines[1] have stated the salient feature in the study of dinitrophenol is the negative findings in so many phases of its metabolic action.

The administration of a different oxidative stimulant, thyroid extract, to partially depancreatized dogs results in a severe and even fatal diabetes,[10, 11] and similarly, thyroid medication, or the spontaneous occurrence of hyperthyroidism, greatly increases the severity of human diabetes, inhibiting the action of insulin and often precipitating ketosis.[12] In the past the generally accepted explanation of the adverse effect of hyperthyroidism on diabetes mellitus has been that advanced by Wilder,[12] namely that with the important exception of exercise, "measures or conditions which stimulate the general metabolism have a depressing action on the tolerance of the diabetic patient."

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[1] Tainter, M. L., Cutting, W. C., and Hines, E., *J. Pharm. and Exp. Therap.*, 1935, **55**, 326.

[2] Hall, V. E., Field, J., Sahyun, M., Cutting, W. C., and Tainter, M. L., *Am. J. Physiol.*, 1933, **106**, 432.

[3] Cutting, W. C., and Tainter, M. L., *J. A. M. A.*, 1933, **101**, 2099.

[4] Dunlop, D. M., *Brit. Med. J.*, 1934, **1**, 524.

[5] Furth, O., and Rapoport, S., *Biochem. Z.*, 1934, **272**, 81.

[6] Magne, H., Mayer, A., and Plantefol, L., *Ann. de Physiol. et de Physiochim. Biol.*, 1932, **8**, 51.

[7] Magne, H., Mayer, A., and Plantefol, L., *Ann. de Physiol. et de Physiochim. Biol.*, 1932, **8**, 71.

[8] Hall, V. E., Brown, C. A., and Sahyun, M., Proc. Soc. Exp. Biol. and Med., 1933, **31**, 380.

[9] Muntwyler, E., Myers, V. C., Danielson, W. H., and Zorn, C., *Am. J. Physiol.*, 1935, **113**, 186.

It appeared to us worthwhile to test this explanation by studying the effect on diabetes of the markedly increased metabolism resulting from the administration of dinitrophenol to the depancreatized dog maintained on insulin. If hypermetabolism *per se* increases the severity of diabetes an augmented glycosuria might be expected from this procedure, and the greatly increased oxidation of fat in the presence of a limited carbohydrate consumption, should, according to generally accepted ideas, favor the development of ketosis. The entirely negative outcome of the experiment about to be described, together with its lengthy and painstaking character discouraged us from extending our observations to more than the one dog. We believe, however, that the experiment is of sufficient interest to warrant a brief description, especially in view of 2 recent reports which lead to similar conclusions. Wishnofsky and his coworkers[13] observed that the administration of dinitrophenol to human diabetics did not increase their hyperglycemia either in the fasting state or after the ingestion of dextrose, and that the glycosuria was decreased by the drug. These experiments were of two hours' duration. Barker[14] recently reported that the excretion of ketone bodies by completely diabetic dogs was not augmented by the markedly increased oxidation of fat resulting from hypermetabolism induced by the administration of dinitrophenol. Apparently the adverse effect of spontaneous or experimental hyperthyroidism on diabetes is not due solely to the increased metabolism which accompanies that condition, and dinitrophenol produces no striking alteration of the metabolic abnormalities of diabetes mellitus.

The male dog used in this experiment was completely depancreatized in November, 1935, and the observations were made in May

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[10] Allen, F. M., *J. Metabol. Res.*, 1922, **1**, 619.

[11] Shpiner, L. B., *Am. J. Physiol.*, 1930, **92**, 672.

[12] Wilder, R. M., *Arch. Int. Med.*, 1926, **38**, 736.

[13] Wishnofsky, M., Kane, A. P., Shlevin, E. L., and Byron, C. S., *Arch. Int. Med.*, 1935, **56**, 374.

[14] Barker, S. B., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 893.

of 1936. In January he was placed on a weighed diet of beef heart, sugar, raw pancreas, cod liver oil and salt mixture offered in 2 feedings with 15 units of insulin at each feeding and this regime remained unchanged throughout the observation. For the 3 months before the experiment his diabetic condition was quite constant and he excreted from 15 to 30 gm. of glucose a day. During this time his food was all consumed and his weight did not vary.

Metabolism experiments[15] were made in the fasting state in the morning before the administration of insulin or dinitrophenol. The expired air was collected in a spirometer and analyzed with a Haldane apparatus. Analyses were done in triplicate, and each respiratory quotient and total metabolism in Table I represents the average of 2 or 3 ten-minute periods. The dog was trained to lie quietly and the constancy of the oxygen consumptions and respiratory quotients is evidence of satisfactory technic. The urine was collected between catheterizations. Urinary sugar was determined by the Somogyi-Shaffer-Hartmann method applied to 1/100 dilutions of urine treated by the Somogyi zinc precipitation method. Experiments with yeast showed that in this dilution the non-fermentable reducing substances in the urine did not cause a significant error. Urine total nitrogen was determined by the macro Kjeldahl method,

TABLE I.
Effect of Dinitrophenol on a Depancreatized Dog Maintained on Insulin.

			Urine					
Date	Weight, Kg.	Dinitrophenol, Mg.	Sugar, G/24H	Organic Acids, MEq/24H	Total N G/24H	Creat., G/24H	Total R.Q.	Basal Metabolism, Cals./H
5/13			27.4	35	8.6	.45	.70	21.8
5/14	11.7		41.1	44	9.6	.46	.68	23.5
5/15			36.8	40	9.3	.46	.68	21.9
5/16			18.6	34	8.8	.42		
5/17			19.9	50	8.9	.41		
5/18	11.7		34.3	44	9.9	.43	.66	22.6
5/19		/30	43.8	39	9.8	.40	.69	21.6
5/20		30/30	27.3	35	9.2	.39	.69	22.6
5/21	11.6	60/60	27.5	50	8.8	.42	.68	22.1
5/22	11.5	60/60	23.5	33	8.6	.41		
5/23	11.5	60/60	16.1	42	8.4	.45		
5/24	11.6	90/90	21.2	29	7.9	.43		
5/25	11.5	90/60*	17.2	39	8.9	.43	.70	23.3
5/26	11.6	120/120	17.1	30	7.0	.37	.72	21.2
5/27	11.7	120/60	17.9	80	8.7	.41	†	†
5/28	11.6	60/60/60	17.7	34	8.6	.41	†	†

*This dose and all subsequent were administered as the sodium salt of dinitrophenol.

†See Table II.

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[15] Boothby, W. M., and Sandiford, I., *Laboratory Manual of the Technic of Basal Metabolic Rate Determinations,* Philadelphia, 1920.

TABLE II.
Effect of Dinitrophenol on Metabolism of a Depancreatized Dog.

Date	Time	Dinitrophenol, Mg.	Total R.Q.	Metabolism, Cals./H
5/27	8:15 A.M.	120		
	10:00		.73	45.1
	11:00		.73	59.7
	11:15 P.M.	60		
5/28	8:30 A.M.	60		
	10:24		.68	34.2
	10:55		.71	32.0
	11:55		.71	28.4

creatinine by the Folin method, and organic acids by the method of Van Slyke and Palmer. These procedures were all carried out as described by Peters and Van Slyke.[16]

The results are presented in Tables I and II. In Table I, column 3 gives the morning and afternoon dose of dinitrophenol which was administered in capsules with the food. On the afternoon of 5/25 and for the rest of the experiment we changed to the sodium salt of dinitrophenol administered the same way to see if the drug was more effective in that form. Calculation of the nitrogen balance, allowing for the fecal nitrogen 10% of the food nitrogen, shows that for the first 9 days the dog lost 1.1 gm. N a day, and for the last 7 days he was practically in balance (a loss of 0.2 gm. N per day).

It will be noted that up to the last 2 days the metabolism, determined in the morning, was not elevated. That this was because the effect of the evening dose of the drug had worn off by morning, about 15 hours later, is shown by Table II, in which on the last 2 days of the experiment the metabolism was determined at intervals *after* the administration of sodium dinitrophenol. Table II indicates that the doses used could cause a significant elevation of metabolism for a period of hours. The effect of the drug may have been prolonged to some extent by our habit of giving it with the food, but we were unable with 2 doses a day to produce a constant elevation such as occurs in the human, possibly because dinitrophenol is more rapidly destroyed and excreted by the dog. The negative character of the experiment in all other respects is shown in Table I.

Summary. The administration of dinitrophenol and its sodium salt was without effect on the fasting total respiratory quotient, urinary sugar, organic acids, total nitrogen and creatinine of a depancreatized dog maintained on a constant diet and with insulin.

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[16] Peters, J. P., and Van Slyke, D. D., *Quantitative Clinical Chemistry, Vol. II, Methods,* Baltimore, 1932.

The metabolic effect of the drug, apparent for some hours after its administration, had always disappeared by the time the metabolism was determined in the morning about 15 hours after the last dose. The significance of these results is discussed earlier in the paper.

9432

Relationship Between Peptone Shock and Anaphylactic Shock.

Franklin B. Mead, Carl A. Dragstedt and Simon W. Eyer.
From the Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago.

We have recently reported that the intravenous injection into dogs of a solution of peptone produces shock indirectly by leading to the liberation of a vasodepressor substance, identified as histamine, from the tissues.[1] Thus the mechanism of peptone shock is apparently similar to that of anaphylactic shock.[2] Biedl and Kraus[3] pointed out the remarkable similarities between these conditions. They also reported that peptone shock desensitizes an animal to anaphylactic shock and *vice versa.* They concluded that the fundamental mechanism concerned in these reactions was identical. They interpreted the reactions as indicating that peptone contains some active substance, such as Popielski's vasodilatin, which can produce shock directly, and that in the anaphylactic experiment a mother substance is formed as the result of the sensitization which then discharges this active peptone constituent when the shocking injection of antigen is made. Subsequent investigators have had variable results in attempting to influence an anaphylactic reaction by the prior administration of peptone. Hill and Martin[4] in their review summarize the reports as follows: "In short, there is some, but not striking evidence that peptone may inhibit shock within certain defined limits."

In view of our demonstration that both peptone shock and anaphylactic shock are reactions that are brought about indirectly by the liberation of histamine from the tissues of the shocked animal, the relationship between these reactions has considerable significance. For example, the question whether desensitization is related to a depletion or exhaustion of the mobilizable store of histamine in the tissues is subject to direct approach by such a study. The present investigation, therefore, was undertaken in the effort to determine whether or not one reaction would desensitize to the alternative reaction.

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[1] Dragstedt, C. A., and Mead, F. B., *J. Pharm. and Exp. Therap.*, 1937, **59**, 429.

[2] Dragstedt, C. A., and Mead, F. B., *J. Pharm. and Exp. Therap.*, 1936, **57**, 419.

[3] Biedl and Kraus, *Wien. Klin. Wchnschr.*, 1909, **22**, 363.

[4] Hill, J. H., and Martin, L., *Medicine*, 1932, **11**, 141.

Dogs were sensitized by the injection of 5 cc. of horse-serum intravenously and 5 cc. subcutaneously. After incubationary periods of 14 or more days, they were anesthetized with ether and barbital, the carotid cannulated for recording blood pressure, and the femoral vein exposed for injections. In 20 animals anaphylactic shock was provoked by the intravenous injection of horse serum, and then, after recovery peptone was injected. Definite shock reactions occurred in all, 5 being fatal. In 16 animals peptone shock was induced first and the specific anaphylactic reaction second. Shock occurred in all but 3.

It is quite apparent from the results obtained that crossed desensitization between peptone shock and anaphylactic shock is not complete in either direction. Thus it is obvious that desensitization is not accounted for on the basis of an exhaustion of the available supply of histamine in the tissues. We have been able to demonstrate the presence of histamine in the blood following the second type of reaction although it had previously appeared and then disappeared as the result of the preceding reaction. It is also indicated that anaphylactic shock is not brought about by the formation of an intermediary substance which exists preformed in peptone solutions since if such were the case crossed desensitization should be as complete as uncrossed. There is some indication that preceding reactions of one kind may diminish slightly the severity of the alternative reaction. The peptone-anaphylaxis experiment is somewhat comparable to a multi-sensitization experiment in which an animal has been simultaneously sensitized to several antigens and the subsequent injection of one antigen decreases but does not abolish the response to the remaining antigens. It does not seem unreasonable to suppose that the diminution in severity of such reactions may be related to a reduction in the available supply of histamine in the tissues but complete homologous desensitization can not be explained upon such a basis.

9433

Effect of Prontosil and Prontylin on Metabolism of Bacteria.

E. S. Guzman Barron and Henry R. Jacobs.

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Donagk discovered[1] that certain sulfonamide compounds are good therapeutic agents against certain bacterial infections, a discovery confirmed by a number of investigators; but the mechanism of this action has not yet been explained.

The bactericidal properties of the reduced sulfonamide compounds, as shown by the inhibition of bacterial growth in cultures, have been reported (Fourneau and coworkers,[2] Colebrook, Buttle, and O'Meara,[3] Long and Bliss[4]). Since the oxidized forms (Prontosil, Prontosil soluble) were found devoid of bactericidal properties, it has been assumed that these drugs become active by their reduction in the animal body (Bliss and Long[5]).

The effect of Prontosil (4-sulfonamide, 2,4'-diamino azobenzol) and of Prontylin* (*p*-amino benzene sulfonamide) on the metabolism of hemolytic streptococci, *B. coli, B. Friedlander,* and gonococci was studied in an endeavor to clarify the mechanism of their chemotherapeutic properties. The experiments were performed at 38° with the usual Warburg-Barcroft respiration apparatus. The bacteria were washed in 0.9% NaCl and suspended in the same salt solution, which was buffered to pH 6.87 by addition of phosphates. The concentration of the drugs was 0.01 M per liter; that of metabolites, 0.008 M per liter. Each experiment was done in quadruplicate, the maximum error being 5%. Prontosil had no effect on the metabolism of these bacteria. Prontylin had a slight inhibiting effect on the oxidation of glucose by hemolytic streptococci, and on the oxidation of glucose and lactate by *B. Friedlander* (Table I).

Prontosil, which is irreversibly reduced by $Na_2S_2O_4$, has an apparent reduction potential of +0.100 volt ±0.01 at pH 6.93. As

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[1] Donagk, G., *Angew. Chem.*, 1935, **48**, 657; *Deutsch. Med. Woch.*, 1935, **61**, 256; *Klin. Woch.*, 1936, **15**, 1585.

[2] Fourneau, E., Trefouel, J., Nitti, F., and Bovet, D., *Compt. rend. Soc. Biol.*, 1936, **122**, 652.

[3] Colebrook, L., Buttle, G. A. H., and O'Meara, R. A. Q., *Lancet*, 1936, **2**, 1323.

[4] Long, P. H., and Bliss, E. A., *J. Am. Med. Assn.*, 1937, **108**, 32.

[5] Bliss, E. A., and Long, P. H., *Bull. Johns Hopkins Hosp.*, 1937, **60**, 149.

* Prontosil and Prontylin were kindly supplied to us by the Winthrop Chemical Co.

TABLE I.
Effect of Prontosil and Prontylin on Metabolism of Bacteria.

Bacteria	Metabolite	O_2 consumption—cmm. per hour			Inhibition, %
		Control	Prontosil	Prontylin	
Hemolytic streptococcus	Glucose	127.3	125.1		None
				117.3	7.9
B. coli	”	655.1	673.2		None
				642.1	”
”	Pyruvate	713.4	752.4		”
				732.6	”
Gonococci	Glucose	579.5	597.7		”
				609.5	”
”	Pyruvate	667.5	650.0		”
				602.0	9.8
B. Friedlander	Glucose	106.2		93.3	12.1
	Lactate	141.4		108.4	23.3

there exist in the animal body a number of oxidation-reduction systems of more negative potential, Prontosil might be easily reduced in the body. *In vitro,* however, Prontosil is not reduced by glutathione at pH 7.31 (temp., 25°).

9434 P

Assay of Progesterone by a New Method.

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Heretofore all the methods used for the assay of progesterone are based upon morphological changes in the uterine mucosa. Usually immature or mature rabbits have been employed. Suitable sensitization of the uterus has been obtained either by the injection of an oestrus-producing hormone (oestrone) or by using rabbits in spontaneous heat. The present communication, however, deals with preliminary experiments on a "physiological" method of assaying progesterone, *i. e.,* by determining the dose necessary to cause contraction of the non-pregnant cat's uterus in response to sympathetic stimulation.

Ovulation and corpus-luteum formation in the cat, as in the rabbit, take place after copulation but not spontaneously so that the ovaries of isolated non-pregnant adult cats do not contain corpora lutea. A further peculiarity of the cat's uterus is the change in its response to hypogastric stimulation or epinephrine as a result of pregnancy. Dale[1] and Cushny[2] independently showed that sympathetic stimulation in the non-pregnant cat causes inhibition of movements and relaxation whereas in the pregnant cat similar stimulation is followed by a uterine contraction. van Dyke and Gustavson[3] demonstrated that such a pregnancy-reversal in response could be elicited in normal or ovariectomized cats receiving both oestrin and corpus luteum extract but not after the injection of oestrin alone. All subsequent work indicated that the pregnancy-response of the cat's uterus can be obtained only if the cat's ovaries contain corpora lutea or if corpus luteum hormone has been injected.

It is not known what is the importance of sensitizing the uterus by first injecting oestrus-producing hormone. However, on the assumption that it is important in facilitating and possibly opposing the pregnancy-like reversal in response due to progesterone, I have tentatively adopted the procedure here outlined. Adult normal cats, isolated long enough so that pregnancy is ruled out, receive the following course of injections:

Day 1: 0.05 mg. (500 international units) of oestradiol benzoate.
" 2: " " " " " " " "
" 4: One third of the total dose of progesterone.
" 5: " " " " " " " "
" 6: " " " " " " " "
" 7: Determination of the effect of intravenously injected epinephrine (usual minimally effective dose about $1\text{-}3\gamma$) on the uterine movements about 30 hours after the last injection of progesterone.

The ovaries were intact in all the cats except 4 which had been ovariectomized about a week before assay was started. The hormone-preparations, dissolved in olive oil, were administered subcutaneously once daily. For the final "acute" experiment the barbiturate "Evipan Na" was found to be an extremely satisfactory anesthetic (70 mg. per kilo intraperitoneally). The preliminary results obtained are given in Table I.

A large dose[4] of oestradiol benzoate was purposely chosen so that if cats with intact ovaries are used, spontaneous secretion of follicular hormone probably does not introduce much variation in the follicular hormone phase of the assay. One experiment suggests that such a dose of oestradiol benzoate suppresses the secretion of

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[1] Dale, H. H., *J. Physiol.*, 1906, **34**, 163.

[2] Cushny, A. R., *J. Physiol.*, 1906, **35**, 1.

[3] van Dyke, H. B., and Gustavson, R. G., *J. Pharm. and Exp. Therap.*, 1929, **37**, 379.

[4] Rowlands and McPhail (*Quart. J. Exp. Physiol.*, 1936, **26**, 109) concluded that 10,000 I.U., administered over 10 days, is a suitable sensitizing dose of *oestrone* for the adult cat.

TABLE I.
Pregnancy-Response of Normal Cat's Uterus After Injection of Oestradiol Benzoate[5] and Progesterone.[5]

Progesterone (total dose) mg.	Av. wt. of cats kg.	No. of cats	Pregnancy-response +	Pregnancy-response —
0	1.95	4	0	4
0.25	1.83	3	0	3
0.50	1.95	13	8	5*
0.50	2.29	4†	2	2
0.75	2.04	2	1	1
1.00	2.25	3	3	0

[5] I am indebted to Schering-Kahlbaum A. G., of Berlin, who supplied me with crystalline samples of both hormones.

*Including one experiment in which contraction occasionally occurred if the uterine tone was low.

†Ovariectomized cats.

the anterior pituitary hormone which maintains the corpora lutea. In one cat there was an early pregnancy discovered only at experiment. All the corpora lutea were clearly atrophic; all the embryos were undergoing necrosis. The uterus of this cat usually relaxed after the injection of epinephrine.

9435

Nutritive Value of Rice Bran Extract for Cultivating Microörganisms.

SAM. C. WONG. (Introduced by C. E. Lim.)

From the Department of Bacteriology and Immunology, Peiping Union Medical College.

Rice bran has been implicated by various investigators to contain growth-stimulants. Williams and his associates[1] claimed that pantothenic acid present in the rice-bran extract is chiefly responsible for the growth stimulation of certain strains of yeast. Salle and Dunn[2] working with *Escherichia coli* found that the extent of growth was related directly to the concentration of rice-bran extract added to meat-infusion broth. *Alcaligenes fecalis* did not behave in the same manner. They concluded that the enhancement of growth in the case of *E. coli* was due to the presence of a growth-promoting substance. The presence of vitamin B in rice bran has long been recognized. West and Cruz[3] found that in addition to vitamin B, vitamin E is also present. While the existence of such growth-stimulating substances may well be admitted, it is of interest to determine whether rice-bran extract by itself will serve as an efficient nutrient for cultivating microörganisms.

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[1] Williams, R. J., and Associates, *J. Am. Chem. Soc.*, 1933, **55**, 2912; *Biochem. J.*, 1934, **28**, 1887; *Science*, 1935, **82**, 178.

[2] Salle, A. J., and Dunn, R. W., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 168.

In our present study it was found that the best extract was prepared by macerating 200 gm. of rice bran with one liter of 12.5% alcohol and incubating at 37°C. for 48 hours with occasional shaking. After centrifugation about 800 cc. of extract was recovered. This was concentrated to about 50 cc. either by vacuum-distillation or by evaporation in a steambath. After cooling, the extract was centrifuged to remove coagulated material, adjusted to pH 7.0 with sodium hydroxide, allowed to stand in the icechest for at least half an hour, centrifuged, made up to 85 cc. and sterilized either by filtering through a Seitz filter or by autoclaving at 10 pounds pressure for 30 minutes. In the latter process of sterilization the extract was adjusted to pH 7.4 before autoclaving. The final extract was stored in the icechest.

Low concentrations of alcohol were better solvents for extraction than high concentrations. The results are shown in Chart 1.

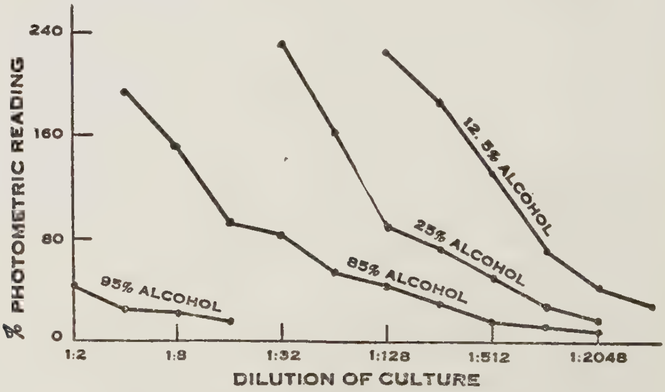

CHART 1.

Growth of *Monilia tropicalis* on rice bran extract prepared by various concentrations of alcohol.

The organism used for the determination of the nutritive substance was *Monilia tropicalis*. This organism was selected after preliminary testing of several species of yeast-like fungi. It was found that all fungi behaved similarly and therefore the results obtained with one were thought to be representative of other similar fungi. The extracts were diluted with distilled water to various concentrations and inoculations were made with the same suspension of *Monilia tropicalis*. After 48 hours of incubation the extent of turbidity produced by the growth in each dilution of the extract was measured by the Pulfrich photometer[4] using the number 4 opacity-glass as a standard of turbidity. A study of Chart 1 shows that the most nutritive portion was obtained with 12.5% alcohol, the poorest with 95% alcohol. In the former extract, significant growth was observed in the dilution of 1/4098 after 48 hours of incubation. Growth was so profuse at the dilution of 1/64 that a direct reading could not be made. In the latter fraction no growth occurred beyond the dilution of 1/16. It might be mentioned that 95% and 85% alcohol were unsatisfactory solvents for extraction because of the large amount of oil extracted from the rice bran. Likewise, concentrations of 6.25% and 3.12% of alcohol were unsuitable because of heavy bacterial contamination.

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[3] West, A. P., and Cruz, A. O., *Philip. J. Science*, 1933, **52**, 1.

A further examination of the chart reveals another interesting point, namely, the quality of the rice-bran extract appears to be related inversely to the concentration of alcohol. In order to show that alcohol plays an important rôle in the extraction the following experiment was carried out. *Monilia tropicalis* was used instead of rice bran because the extracts from the former could be obtained without bacterial contamination, particularly when water or normal saline solution were used as solvents. The organisms obtained were divided into 4 equal portions and treated with (a) dilute ethyl alcohol, (b) boiling water, (c) distilled water, and (d) normal saline. The various extracts in different concentrations in distilled water were inoculated with *Monilia tropicalis* and incubated at 37°C. for 48 hours. The results showed that the amount of nutritive material extracted by boiling water, distilled water, and normal saline was approximately the same while that by the dilute alcohol was many times superior. Significant growth was observed in the dilution of 1/1024 in the alcoholic portion while no growth occurred beyond the dilution of 1/256 in the other portions.

A comparative study of the alcoholic extracts of various vegetable substances such as rice bran, wheat bran, oat bran, freshly grown cultivated mushrooms, and soy bean was made to determine which was the most nutritive. Inoculation of the various dilutions was made with *Monilia tropicalis*. Meat-infusion broth was included as a control. After 24 hours of incubation at 37°C. it was found that the best result was obtained with rice-bran extract while wheat bran, oat bran, mushroom, soy bean, and meat-infusion broth followed in the order named. Growth of *Monilia tropicalis* was not observed beyond the dilution of 1/128 in the case of meat-infusion broth while growth was observed in all others in the dilution of 1/1024.

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[4] Kurotchkin, T. J., and Lim, C. E., *Chinese Med. J. Suppl.*, 1936, **1**, 261.

Comparative studies were made with rice-bran extract and meat-infusion broth with the following cultures: *Escherichia coli, Eberthella typhi, Salmonella paratyphi, Salmonella schottmulleri, Salmonella hirschfeldi, Salmonella enteritidis, Salmonella aertryke, Shigella dysenteriae,* and *Staphylococcus aureus.* The results indicated that rice-bran extract was equal to or better than meat-infusion broth for growing these bacteria. The organisms grown on rice-bran extract were also found to be suitable for serologic agglutination. Of the organisms we have studied, any that can be cultivated on meat-infusion broth can also be grown with equal success on this rice-bran extract.

Chemical analyses of the extract prepared with 12.5% alcohol for total nitrogen by the micro-Kjeldahl method and for reducing substance by the Hagedorn and Jensen[5] method showed that one cc. of the extract contained 5 mg. of nitrogen and 130 mg. of reducing substance. One per cent Bacto-peptone was found to contain 1.1 mg. N per cc. In other words the rice-bran extract is equal to about 5% peptone in nutritive value if the total nitrogen is used as a standard of comparison. It may be stated that the rice-bran extract gave none of the common reactions for protein. The presence of glucose was shown by the osazone test. The whole rice-bran extract was precipitated with 95% alcohol, or anhydrous acetone, or 85% acetone, thereby dividing it into 3 soluble and 3 insoluble fractions. Total nitrogen and reducing substance on these fractions were determined and then the soluble and insoluble fractions in various concentrations were tested with *Monilia tropicalis* and *E. coli* either by themselves or by adding them to a synthetic medium. Since the extent of growth obtained was in accord with the chemical analyses on these 6 fractions, it is sufficient to state that any claim for the stimulation in growth of these microörganisms may be accounted for by the amount of nutritive materials present.

A suitable method is recorded for the extraction of nutritive materials from rice bran. This extract was found to be equal to or better than meat-infusion broth for growing many microörganisms.

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[5] Hawk and Bergheim, *Practical Physiological Chemistry,* 19th Edition, 1931.

9436

Salmonella enteritidis Infection by a Parenteral Route.

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In the spring of 1936 a small epidemic of *enteritidis* bacteriemia was reported from refugee camps in Peiping.[1] As the majority of these patients suffered from relapsing fever at the same time, and as *enteritidis* bacteriemia has in the past rarely been encountered locally,[2] an attempt was made to see whether there was a special route of infection. Body-lice caught from some of the patients were cultured and, in every case examined, they were found to be infected with *S. enteritidis*. In several cases, both *Spirochæta recurrentis* and *S. enteritidis* were found in the same ectoparasites. There was also evidence of multiplication of the infecting organisms in the body-lice by repeated cultures of the infected lice at different intervals. In one experiment clean body-lice were fed with blood from a patient who was found to have about 150 colonies of *S. enteritidis* per cc. of circulating blood. Lice that were fed on the patients were found to contain innumerable colonies of the same organisms. In view of the susceptibility of body-lice to infection with *S. enteritidis* and in view of the occurrence of combined infection with *Spirochæta recurrentis* both locally and in Russia,[3] it seemed to us that it was not impossible that the 2 infections were introduced together by a parenteral route. With this idea in mind, the following experiment was undertaken on guinea pigs, which are naturally susceptible to *enteritidis* infection.

Guinea pigs were shaved over the nape with care so that no gross trauma was caused. After 24, 48 and 72 hours, animals were infected by repeated but gentle swabbing of this area with a 24-hour broth culture of *S. enteritidis*. Some animals were purposely traumatized shortly before the application of the culture. After the swabbed area appeared dry, it was covered by a large piece of adhesive plaster. In addition, unshaven controls were kept in the same cages in order to see whether there was possibility of infection by mouth. Body-temperature was taken daily and stool and blood cultures were frequently made. When an animal died, complete bacteriologic examination of the body was done.

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[1] Huang, C. H., Chang, H. C., and Lieu, V. T., *Chinese Med. J.* In press.

[2] Wu, C. J., and Zia, Samuel H., *Chinese Med. J.*, 1935, **49**, 1217.

[3] Hicks, E. P., *J. Hyg.*, 1930, **29**, 446.

As the results of several experiments were identical, those of only one are given. All guinea pigs shaved but not traumatized acquired *enteritidis* bacteriemia in 3 to 15 days. Longer incubationary periods were observed in those in which the intervals between shaving and swabbing were longer. One traumatized animal got fever on the second day. All these animals gave positive blood and stool cultures, both before and after death. Two controls showed no fever and stool cultures were always negative. They were discarded after one month's observation.

The results seem to show that bacteriemia with *S. enteritidis* may be readily produced in guinea pigs through a parenteral route with even a relatively small dose. The rapidity of infection seems to be related to the degree of trauma, and the more severe this is, the more rapidly the infection is produced. Animals with no apparent trauma may require an incubationary period of 15 days before fever appears. This brings *S. enteritidis* in line with *Br. melitensis,* [4, 5] yellow-fever virus,[6] and *Mycobacterium tuberculosis,*[7] in ability to pass through reasonably intact skin and to set up infection, which may explain the mode of infection in the cases of bacteriemia which have occurred in local epidemics.

9437

Pathogenicity of Different Types of *C. diphtheriæ* for Chinese Hamsters.

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Since Anderson, *et al.,*[1] described 3 types of *C. diphtheriæ,* the relative virulence of these organisms for common laboratory animals has been the subject of much study. Povitsky, Eisner, and Jackson[2] observed that it took 4 times smaller dose of *gravis* than *mitis* types to kill guinea pigs. Murray[3] showed that in virulence for rabbits,

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[4] Hardy, A. V., Hudson, M. G., and Jordan, C. F., *J. Inf. Dis.*, 1926, **45**, 271.

[5] Eyre *et al., Report Mediterranean Fever Committee,* 1906-1907, Part VI, p. 44.

[6] Bauer, J. H., and Hudson, N. P., *Am. J. Trop. Med.*, 1928, **8**, 371.

[7] Ono, I., *Kekkaku Abstr. Sect.*, 1935, **13**, 20.

[1] Anderson, J. S., Happold, F. C., McLeod, J. W., and Thomson, T. G., *J. Path. and Bact.*, 1931, **34**, 667.

[2] Povitsky, O. R., Eisner, M., and Jackson, E., *J. Inf. Dis.*, 1933, **52**, 246.

[3] Murray, J. F., *Brit. J. Exp. Path.*, 1935, **16**, 384.

the order was *gravis, intermediate, mitis.* Gundel and Ezrin[4] found that the organisms of the *gravis* type were not only more virulent than those of *intermediate* and *mitis* types, but definitely more invasive in guinea pigs. However, Parish and his coworkers[5] could demonstrate no such high virulence of the *gravis* type either in rabbits or in guinea pigs. In view of such conflicting reports, a further study of this problem in another species of laboratory animal seems to be desirable. As the Chinese hamsters have been found to be highly susceptible to *C. diphtheriæ* infection, and since they can be easily procured in large numbers and at low cost,[6] these animals have been chosen for the present study.

Eight strains of *gravis,* 5 of *intermediate,* and 8 of *mitis* types were employed. Two of each type were kindly supplied by Professor McLeod of the University of Leeds and the rest were isolated locally from cases of diphtheria. Eighteen-hour Loeffler's slant-cultures were emulsified in 2 cc. normal saline and shaken vigorously in order to produce an even suspension. An approximately equal concentration of organisms was obtained by diluting the different cultures to an equal opacity. These suspensions were then diluted 10, 100, and 1000 times with normal saline. Two-tenths cc. of the original suspension and of each dilution were inoculated intramuscularly into 5 hamsters each weighing from 25 to 30 gm. Animals which did not succumb to the test culture within 9 days were considered survived. The highest dilution of a culture which killed the majority of animals was taken as a measure of virulence of the strain. Recovery of organisms from various internal organs and the thoracic cavity was attempted on those that died from 20 to 72 hours after inoculation of 0.2 cc. of 1-10 or 1-100 dilution. Cultures were made on blood-agar containing 0.02% of potassium tellurite.

Results. It was found that every culture of the 3 types tested was virulent for hamsters at the dilution of 1-100 but none in that of 1-10,000. The only difference in virulence was shown at the dilution of 1-1000 at which a few strains of each type were able to kill the experimental animals.

Of various internal organs and the thoracic cavities from which the recovery of organisms was attempted, it was found that among 72 hamsters the lungs yielded the highest percentage of positive cultures, *viz.,* 22%. Thoracic cavities gave 8%, adrenals 4%, kidneys and testes 3% each and spleen 1.5%. Liver and heart's blood

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[4] Gundel, M., und Ezrin, N., *Zentrab. f. Bakt.,* 1936, **136**, 24.

[5] Parish, H. J., Whatley, E. E., and O'Brien, R. A., *Brit. Med. J.,* 1932, **12**, 915.

[6] Lu, K. J., and Zia, S. H., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 334.

TABLE I.

Virulence of different types of *C. diphtheriæ* in hamsters.

Dilutions	Strains	Gravis								Intermediate					Mitis							
		Leeds 16*	Cork 9*	5173	5337	4435	6441	6580	6695	London*	Hull 121*	Park 8	5016	5456	Hull 136*	Leeds 29*	6079	5275	8232	9317	3510	6520
10–1		5†	—	5	—	—	—	—	—	5	5	5	—	4	5	—	5	—	—	—	—	—
10–2		3	5	5	5	5	5	5	5	5	5	5	5	4	5	5	5	4	5	5	5	5
10–3		4	4	5	0	0	0	0	0	0	3	2	0	0	1	0	5	0	0	0	0	0
10–4		0	0	0	—	—	—	—	—	—	0	—	—	—	—	—	0	—	—	—	—	—

* = strains supplied by Professor McLeod.
†Number = animals died.
0 = no death.
— = not done.

were always sterile. It is interesting to point out that in this study no appreciable difference in invasiveness was noticed in any of the 3 types.

The result of the present study seems to indicate that in Chinese hamsters, at least, the organisms classified as the *gravis* type showed neither greater pathogenicity nor enhanced invasiveness. Whatever difference that was obtained must be attributed to the difference in the individual strain rather than to the difference in types.

9438 P

Specific Carbohydrate from *Asterococcus mycoides* for Serologic Tests of Bovine Pleuropneumonia.

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It is known that a substance active in the specific precipitative reaction is present in the serum of infected animals and can also be extracted from the affected lungs in cases of bovine pleuropneumonia. These observations suggest the presence of a haptenic carbohydrate. An attempt, therefore, was made in the present experiment to isolate a specific carbohydrate from pure cultures of *Asterococcus mycoides,* the causal agent of bovine pleuropneumonia, and to determine its serologic activity with immune rabbit's serum.

The strain of the organism used here was isolated from the pleural exudate of a fatal case of pleuropneumonia in a cow. Forty flasks, each containing 200 cc. of liver-digest broth enriched by addition of 5% unheated horse serum, were inoculated with the organism and incubated for 5 days at 37°C. The growth was centrifuged for 2 hours at 3,000 r.p.m. and the sediment was collected, washed in saline, centrifuged again and finally resuspended in distilled water. For the sediment obtained from one liter of culture 20 cc. of water was employed. This suspension was left in the icechest overnight. At the end of this period, 0.5% of potassium hydroxide was added following which the suspension became transparent and markedly slimy. This was centrifuged and to the clear fluid glacial acetic acid was added. A floccular precipitate, designated here as nucleoprotein, developed. The precipitate was collected, dried in the incubator and preserved for serologic tests. The remaining clear fluid was passed through a Seitz filter and further precipitation accomplished by addition of 5 volumes of 95% ethyl alcohol. The mixture was left in the icechest for 18 hours. During this time a small amount of white precipitate was formed. This was collected, dried in the incubator and used in the serologic tests. Because of the small amount of the substance, the yield of which was 10 mg. per liter of culture, no further purification through reprecipitation with alcohol has been attempted.

In chemical tests the substance gave negative biuret and positive Molisch reactions.

Tests for precipitin and complement-fixation were carried out with rabbit-antiserum prepared by immunizing with washed cultures of *Asterococcus mycoides*. The serum in 1:80 dilution agglutinated the organism. The interfacial test was used to detect precipitin and Kolmer's technic was employed in the complement-fixation test. Undiluted serum was used in the precipitative test, and a 1:5 dilution in the complement-fixation test.

The formation of a distinct ring occurred when the substance was diluted to 1:25,600, while complete inhibition of hemolysis occurred in 1:102,400 dilution of the substance. Nucleoprotein from *Asterococcus mycoides* was also tested serologically. It was found that no precipitation occurred when nucleoprotein was used in a concentration of 1:200, but complete inhibition of hemolysis was observed in dilution 1:6,400. A washed suspension of *Asterococcus mycoides* was also used in the complement-fixation test and positive reactions occurred only when not less than 0.5 cc. of the suspension equivalent in density to the original culture was used.

The data suggest that the substance prepared from pure cultures of *Asterococcus mycoides* is a specific carbohydrate having characteristics similar to those derived from other organisms. The high specific sensitivity of the substance indicates the possibility of its use for diagnostic purposes especially for the detection of chronic cases of bovine pleuropneumonia.

9439

Modus Operandi of Commercial Heparin.

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The full explanation of the clot-inhibiting action of heparin (Howell and Holt's[1] anticoagulant liver extract) has awaited the reconciliation of the conflicting results of various investigators. The present experiments were designed to assist in the search for the experimental variables involved. Three technical points received special attention: (1) adequate controls[2]; (2) the time factor in certain reactions; (3) the effective concentration zones of the chief reagents as measured by a dilution method.

0.5 cc. of a moderately weak thrombin mixture [prothrombin soln. (10) + 0.1% cephalin (1) + N/10 $CaCl_2$(1)] gave solid clots in 1.0 cc. of prothrombin-free dog fibrinogen in 15-60 sec.[2] Successive dilutions of a 1% stock solution of commercial (H. W. & D.'s) heparin were used in the following tests (all performed at 38°C.).

A. A true *antithrombic action* could be demonstrated by adding 1-3 mg. heparin (1:1000 soln.) per one cc. of 'ripe' (*i. e.,* maximally activated) thrombin and subsequently testing its clotting power. As long as the reagents were added simultaneously it was immaterial whether the heparin was added to the thrombin or to the fibrinogen first. In other words the inhibition was 'immediate'. Nevertheless, if the heparin was incubated with the thrombin before the clotting test, the neutralization was definitely enhanced. The rapid and solid clots with control thrombins substantiate Howell's (Cekada[3]) claim that "antithrombin is absent from the solutions of prothrombin prepared by the acetone method." There is nothing to suggest that this antithrombic action of heparin is other than direct. A flocculation of fibrinogen by the heparin proved troublesome in some of the tests but it was found possible to prevent this by controlled alkalinization with N/10 KOH.

B. 0.2-0.1 mg. heparin per 1 cc. of thrombic mixture (proved by control tests *(A.)* to lack enough direct antithrombic action to prevent the formation of good clots) were able completely to inhibit the elaboration of thrombin from the prothrombin, cephalin, calcium mixture. Whereas the control 'activation curve' (*sine* heparin) reached a steady level (maximal activation) in some 5-10 min., the series containing the weak heparin solutions were not active even after 60-90 min. This is the classical *antiprothrombic* action of Howell.[1]

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[1] Howell, W. H., and Holt, E., *Am. J. Physiol.,* 1918, **47**, 328.

[2] Ferguson, J. H., *Am. J. Physiol.,* 1936, **117**, 587.

[3] Cekada, E. B., *Am. J. Physiol.,* 1926, **78**, 512.

C. Thrombic mixtures (v. supra) were incubated with varying dilutions of heparin until the minimal effective zone for the antiprothrombic effect was reached. The test was then repeated with stronger cephalin (1%) added to the mixture. The diminution of the antiprothrombic action of heparin as the result of the extra *cephalin* added is clearly seen in Table I.

TABLE I.

A. Composition of Thrombic Mixtures (incubated at 38°C.).

	Prothrombin cc.	Dist. Water cc.	Cephalin cc. %	Heparin cc.	N/10 $CaCl_2$ cc.
I	5	1.0	.5 (0.1)	—	.5
II	5	—	.5 (0.1)	1.0 (1:1000)	.5
III	5	0.5	.5 (0.1)	0.5 (1:3000)	.5
IV	5	0.5	.5 (0.1)	0.5 (1:1000)	.5
V	5	—	.5 (0.1) .5 (1.0)	0.5 (1:1000)	.5

B. Clotting-times for Prothrombin-free Fibrinogen (38°C.).

Age of mixture		15 sec.	1 min.	5 min.	10 min.	20 min.	30 min.	60 min.
Mixture	I	15¾ min.	6¾ min.	45 sec.	25 sec.	20 sec.	25 sec.	45 sec.
"	II	∞	∞	∞	∞	∞	∞	∞
"	III	480 sec.	490 sec.	145 sec.	95 sec.	40 sec.	45 sec.	45 sec.
"	IV	Overnight	Overnight	—	55½ min.	35 min.	25 min.	5 min.
"	V	4 hr.	4 hr.	—	75 sec.	40 sec.	40 sec.	40 sec.

D. Regarding the ability of cephalin to neutralize the direct antithrombic action of our heparin solutions, the results were less conclusive. If clotting was completely prevented, a moderate excess of cephalin over heparin (dry weights) did not appear to affect the result. However, in all cases in which the heparin merely delayed coagulation, the tubes containing extra cephalin showed quicker and better clots. Controls proved that no increase in the potency of the thrombin itself resulted from the additional cephalin.

These preliminary data are offered with no intention of relying on them for any theory as to the rôle of heparin in blood coagulation. They merely serve to indicate the type of tests to which any prepared anticoagulant should be subjected. It is highly probable that inconsistencies in the experimental literature are chiefly the result of working with quantities of the respective reagents without regard to the zone of minimal effective amounts. A large excess of potent thrombin might readily mask any antithrombic action. The phospholipid antagonisms, in our experience, are difficult to demonstrate except with limiting dilutions of heparin. The dilution method permits experimentation in the zone of minimal quantities of reagents which we are still unable to isolate and work with in pure chemical form.

9440 P

Reflex Inhibition of the Human Heart: Complete A-V Block and Parasystole.

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Certain phenomena observed in the electrocardiogram have forced the question: May reflex augmentation of inhibitory tone during the systolic rise in arterial pressure produce effects which wax and wane within the time of a single cycle, or are the reflex effects smoothed out so that little change in the degree of inhibition occurs within a single cycle?

The experimental results of Brown and Eccles[1] suggest that the first alternative may be the true one. In their experiments, however, one (or 2) stimuli were applied to the vagus nerve and time was then allowed for full recovery from inhibition before another stimulus was given. If a stimulus be applied every 2 cycles, or less, conditions as they sometimes obtain in the human heart are more closely approximated. A curve illustrating the time course of vagus slowing is shown in Fig. 1. Make-break shocks were applied about every 650σ to the peripheral end of the cut right vagus of a dog under urethane anesthesia. The other vagus was also cut. Electrocardiograms were taken. The uninhibited cycle was 350σ. Several experiments were carried out with similar results.

The curve shown rests upon a background of inhibition resulting from the frequency of nerve stimulation. The zero point on the ordinates is an arbitrary point of reference. The latency of inhibitory effect is 170σ; the ascending limb of the curve is about 300σ.

In several experiments the rate of vagus stimulation was only slightly slower than the inhibited sinus rate. Under these condi-

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[1] Brown, G. L., and Eccles, J. C., *J. Physiol.*, 1934, **82**, 211, 242.

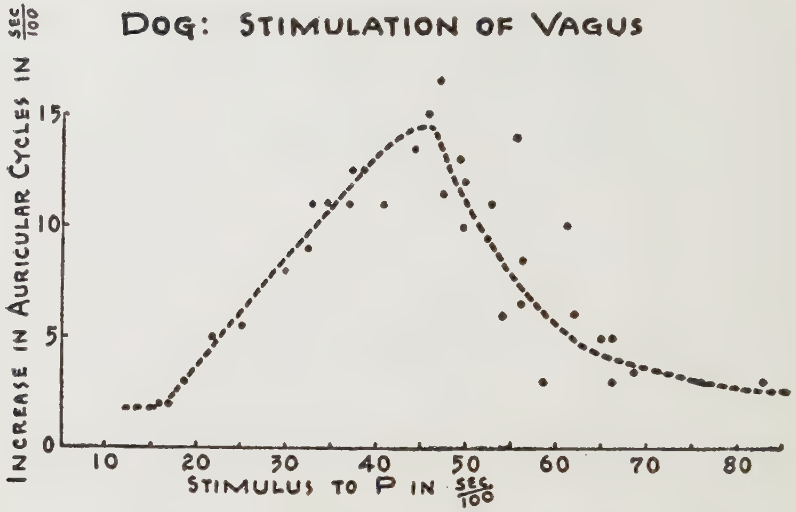

Fig. 1.

tions the heart slowed or accelerated, depending upon the time in the cycle at which the stimuli fell.

Only when the rhythm of the physiological inhibitory reflex pulses differs from the rhythm of the pacemaking structure can a waxing and waning of inhibitory effects be demonstrated in the human heart. This requirement is satisfied in the following examples.

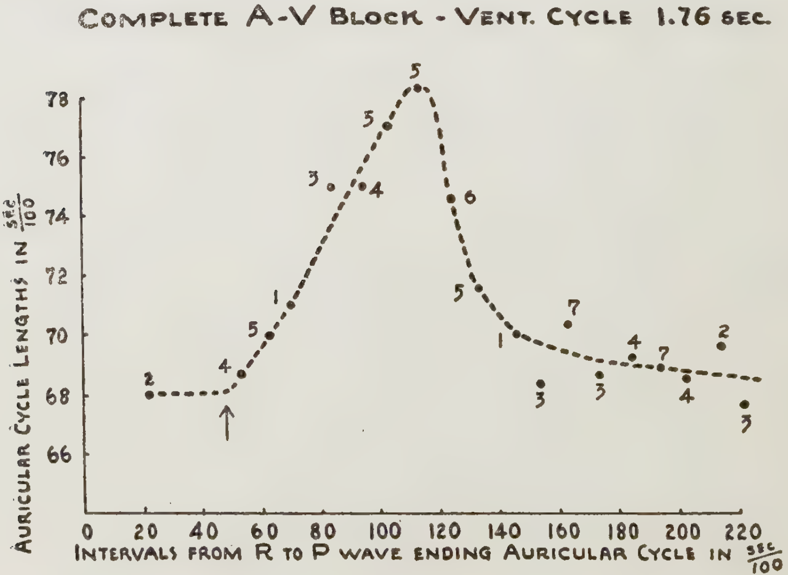

Fig. 2.

Figure 2 is derived from the electrocardiogram of a patient with complete A-V heart-block. Each point is the average of the number of individual measurements shown by the numbers. The reflex latency is 480σ. When corrected for the delay between the electrical and mechanical responses, for the presphygmic period, and for transmission of pressure to aortic nerve receptors, this latency becomes about 370σ. In experiments upon 2 dogs in which reflex slowing of the heart was brought about by raising the pressure in the carotid sinus, the latency was found to be of this order of magnitude or a little longer. The ascending limb of the curve is 700σ. Since the ejection phase lasted about 500σ, a gradual cumulation of inhibition can be assumed.

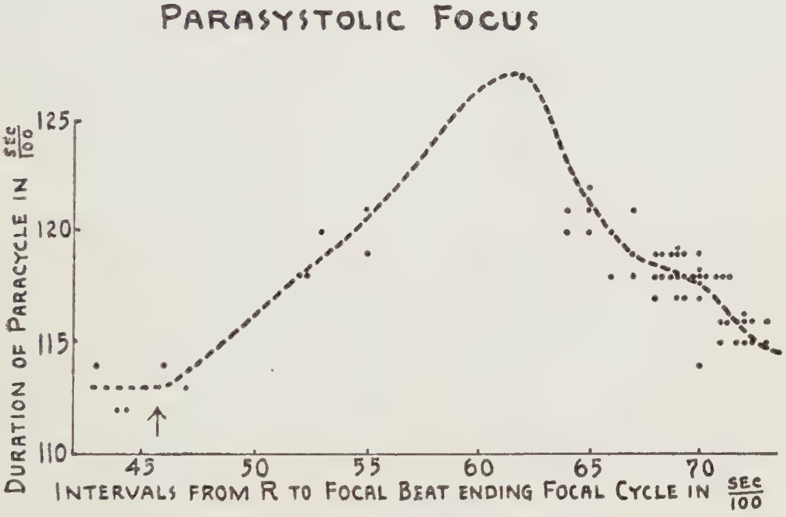

FIG. 3.

Figure 3 is from the case reported in another communication. It illustrates the reflex chronotropic effect upon an ectopic focus of normal systoles interpolated between successive manifest ectopic beats. The latency is 460σ; corrected, about 360σ. Here the ascending limb of the curve is only about 170σ. Possible reasons for its brevity cannot be discussed here.

These results and observations support the view that reflex inhibition of a pacemaker may wax and wane within the time of a single cardiac cycle.

9441

The Effect of Gastric Distention on Gastric Secretion.

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Associated with ileus there is a dehydration and hypochloremia due to the loss of fluids and chlorides in the gastric secretions which normally are reabsorbed. In order to remove these stagnant secretions which produce nausea and vomiting, continuous duodenal drainage is used clinically. An objection raised to continuous duodenal drainage has been that it is likely to increase dehydration and increase the loss of electrolytes.

Herrin and Meek[1] have shown that increased secretion results when the bowel is distended. Raine and Perry[2] observed that distention of the stomach with liquid albolene increased gastric secretion. Lim, Ivy, and McCarthy[3] observed an increased gastric secretion in dogs with stomach pouch preparations when continuous drainage was used. They believed that gastric tone constituted the adequate stimulus to secretion.

The present investigation was undertaken to study the effects of gastric distention on gastric secretion. Thirty-seven dogs were used. Only those surviving long enough for observations are included in the results. In each animal the esophagus was ligated near the cardia with umbilical tape, the jejunum was severed 8 cm. below the ligament of Treitz, and both ends were closed blindly. A cannula of the type described by Owings, *et al.*,[4] was placed in the stomach in order that the gastric contents could be removed at will. The animals were given 70 cc. of Hartmann's solution per kilo of body weight per day. Blood chlorides were determined daily. The volume of each drainage was measured and the activity of each determined.

Fifteen observations were made upon 4 dogs which were drained continuously. The average daily secretion was 133 cc. The acidity of the specimens rose steadily until it was approximately 3 times the normal for these animals. Twenty-eight observations upon 2 animals drained every 6 hours showed an average daily secretion of 78.1 cc. Twenty-four observations on 2 dogs drained every 12 hours showed a daily average secretion of 76.4 cc. Seven observations on 2 dogs drained every 24 hours showed a daily average secretion of 76.2 cc. Thirty-seven observations on 5 dogs drained every 48 hours showed a daily average drainage of 87 cc. In none of the animals except those drained continuously did the gastric acidity rise. The blood chlorides remained within normal limits in all animals. Marked daily variances were observed in the amounts secreted in several of the animals.

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[1] Herrin, R. C., and Meek, W. J., *Arch. Int. Med.*, 1933, **51**, 152.

[2] Raine, F., and Perry, M. C., *Arch. Surg.*, 1929, **19**, 478.

[3] Lim, R. K. S., Ivy, A. C., and McCarthy, J. E., *Quart. J. Exp. Physiol.*, 1925, **15**, 13.

[4] Owings, J. C., McIntosh, C. A., Stone, H. B., and Weinberg, J. A., *Arch. Surg.*, 1928, **17**, 507.

Assuming that there was relatively little gastric distention within 6 hours and that gastric distention occurred after 12 hours, the animals were divided into 2 groups. Group I included those animals drained continuously and every 6 hours. The average daily secretion of 22 observations on 6 animals was 115.8 cc. The second group included those drained every 12, 24, and 48 hours, respectively. Thirty-seven observations upon 9 dogs in this group showed a daily average secretion of 83.4 cc.

It thus appears that in the absence of distention there is an increase in the amount of gastro-duodenal secretions.

Although the clinical use of continuous duodenal drainage is justified because it prevents nausea, vomiting, and abdominal distention, these investigations suggest that when it is used there is an increased gastric secretion and a greater loss of electrolytes.

9442 P

Failure of Ant. Pituitary Extracts to Maintain Fasting Carbohydrate Levels of Hypophysectomized Rats After Preliminary Treatment.*

Leslie L. Bennett. (Introduced by Herbert M. Evans.)

From the Institute of Experimental Biology, University of California, Berkeley.

It has been reported previously that the low muscle glycogen levels of fasted hypophysectomized rats may be maintained at normal or supra-normal levels by the injection of suitable anterior lobe extracts during the fasting period.[1, 2] The present investigation is concerned with the effect of two to three weeks' preliminary treatment with the same standard alkaline extract. Hypophysectomies were performed by the parapharyngeal route and completeness checked at autopsy. All animals were fasted for 24 hours over wire, and analyses were carried out as reported previously.[3] In all cases extracts were injected several times during the fasting period, usually at 24, 20, and 4 hours previous to sacrifice. The extract injected was the same as used previously[1] and is rich in growth hormone besides containing at least traces of all the other known anterior lobe hormones.

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* Aided by the Presnell Fund for the study of the metabolic relations of the anterior hypophysis, contributed by Robert R. Presnell, Frank Tuttle, Arthur Stebbins, and Mrs. Gordon Kahn, of Los Angeles.

In Table I are summarized the results of these experiments. Inspection of the data shows that as small an amount of extract as one mg. per 100 mg. body weight produces complete maintenance of muscle glycogen when it is given only during the fasting period. On the other hand, much larger amounts of extracts are without effect if the fasting period has been preceded by 2 to 3 weeks of treatment. During the period of treatment these hypophysectomized rats grew continuously at an average rate of 3 gm. per day.

Since unfasted hypophysectomized rats which have been treated for 3 weeks show normal carbohydrate levels, the low values in these

TABLE I.
Carbohydrate Levels in 24-hour Fasted Rats.

	Muscle Glycogen in mg. %		Liver Glycogen in mg. %*		Blood Sugar in mg. %	
	Mean	Range	Mean	Range	Mean	Range
Normal rats (12)†	500	480-545	22	13-33	79	60-89
Untreated hypophysectomized rats (11)†	317	251-400	16	10-22	50	41-63
Hypophysectomized rats receiving 17 mg. of S.A.E.‡ only during fast (9)†	522	421-576	16	10-48	53	37-61
Hypophysectomized rats receiving 1 mg. of S.A.E. per 100 gm. body weight only during fast (5)†	506	445-566	16	13-21	52	44-59
Hypophysectomized rats injected with 10 mg. of S.A.E. per day for 10-11 days§ (5)†	388	356-426	13	11-17	60	53-69
Hypophysectomized rats injected with 14 mg. of S.A.E. per day for 22-24 days§ (5)†	342	297-401	12	10-13	58	45-83

*Fermentable reducing substances only.
†Number of animals in the group.
‡Standard alkaline extract.
§Also injected during fasting period.

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[1] Russell, J. A., and Bennett, L. L., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 406.

[2] Fisher, R. E., Russell, J. A., and Cori, C. F., *J. Biol. Chem.*, 1936, **115**, 627.

[3] Russell, J. A., and Bennett, L. L., *Am. J. Physiol.*, 1937, **118**, 196.

fasted animals can only be due to some obscure refractory state against the metabolic effect of the extract, but apparently not against its growth-promoting effect.

Preliminary efforts to detect an inhibitory effect in the serum in chronically treated normal animals have been unsuccessful. However, in confirmation of Black,[4] it has been found that such chronically treated normal rats became resistant to the ketogenic effects of crude anterior lobe extracts. A group of 7 rats during a 72-hour fasting period, while receiving 1.5 cc. per day of extract, excreted an average of 43 mg. of urinary ketone bodies; after one month's daily injection, this fell to 4.6 mg. for the same fasting period while receiving a like amount of extract. An untreated control group of rats excreted 5.3 mg. of ketone bodies during a 72-hour fast.

Summary. Crude anterior lobe extracts are able to maintain the fasting muscle glycogen levels of hypophysectomized rats in an acute test, but are unable to do so when injected chronically. Continued treatment of normal rats with such extracts abolishes their ketogenic effect.

9443 P

Effects of Hypophysectomy and of Anterior Pituitary Extracts on Disposition of Fed Carbohydrate in Rats.*

JANE A. RUSSELL.† (Introduced by Herbert M. Evans.)

From the Institute of Experimental Biology, University of California, Berkeley.

Rapid disappearance of body carbohydrate and correspondingly high rates of carbohydrate oxidation were observed in fasted hypophysectomized rats, and anterior pituitary extracts were found to restore to these animals the ability to maintain their carbohydrate levels.[1, 2, 3] In order to determine whether hypophysectomized rats differed from the normal in their carbohydrate metabolism in the fed state as well as when fasted, and to determine whether anterior lobe extracts acted also under these conditions, the following experiments were performed:

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[4] Black, P. T., *J. Physiol.*, 1935, **84**, 15.

*Aided by the Presnell Fund for the study of the metabolic relations of the anterior hypophysis, contributed by Robert R. Presnell, Frank Tuttle, Arthur Stebbins, and Mrs. Gordon Kahn, of Los Angeles.

†Porter Fellow, 1937-38.

[1] Russell, J. A., and Bennett, L. L., *Am. J. Physiol.*, 1937, **118**, 196.

[2] Fisher, R. E., Russell, J. A., and Cori, C. F., *J. Biol. Chem.*, 1936, **115**, 627.

[3] Russell, J. A., and Bennett, L. L., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 406.

Normal and hypophysectomized rats were fasted, then fed known amounts of glucose. The oxygen consumption, respiratory quotients and N excretion were determined during the next 4 hours. Then the animals were killed and the levels of blood glucose and of liver and muscle glycogen were determined, as were also the amounts of glucose remaining in the alimentary tracts. Identical experiments were performed at the same time on both normal and hypophysectomized rats which had been treated for various lengths of time with standard alkaline extracts of anterior pituitary. Respiratory data and carbohydrate levels were also determined in normal unfed rats, with and without A. P. injections.

It was found in the hypophysectomized rats that 74% of the absorbed glucose was oxidized, as compared to 53% in the normal animals. The hypophysectomized rats stored less carbohydrate than did the normal rats, the difference being accounted for approximately by the increase in the proportion of carbohydrate oxidized. The actual amounts of carbohydrate apparently oxidized were about the same in the 2 series, in spite of the fact that the total oxygen consumption, and the rate of absorption of the fed glucose were much smaller in the hypophysectomized rats. Therefore, as also found previously by others,[4] the proportion of total calories obtained from carbohydrate was higher in the operated animals. Evidently, under the conditions of these experiments, a factor was operating in the normal but not in the hypophysectomized rats which depressed the proportion of carbohydrate being oxidized, even when there was a plethora of carbohydrate available.

The injection of the anterior lobe extracts caused an immediate fall in the apparent rate of oxidation of carbohydrate in both hypophysectomized and normal rats, in the former to levels below the normal. The amount of carbohydrate oxidized was 44% and 31% of the amount absorbed in the hypophysectomized and normal rats respectively, and carbohydrate oxidation accounted for only 39% and 25% of the total calories under these conditions. Much more carbohydrate was stored as glycogen by the normal rats injected with A. P. than in those not treated. This change was not marked in the treated hypophysectomized animals, but the blood sugar levels rose rather higher in the operated than in the normal rats under these conditions.

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[4] Fisher, R. E., and Pencharz, R. I., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 106.

In the normal unfed rats, no action of the A. P. extracts was demonstrable, except a slight increase in the muscle glycogen level. The average R. Q. was 0.722, compared to 0.724 in uninjected rats. Since carbohydrate oxidation was already at such a low level under these conditions, the absence of a further decrease was not surprising.

9444 P

Production of Refractoriness to Action of Anterior Pituitary Extracts in Depressing Oxidation of Fed Carbohydrate.*

JANE A. RUSSELL.† (Introduced by Herbert M. Evans.)

From the Institute of Experimental Biology, University of California, Berkeley.

In the previous paper, it was reported that anterior lobe extracts caused a fall in the apparent rate of oxidation of fed carbohydrate in both hypophysectomized and normal rats. The decrease, judged by the R. Q. and the glycogen deposition after feeding glucose, occurred within a few hours after the injection of standard alkaline extracts, and was also evident when injections had been made over several days preceding the experimental periods. It was found recently in this laboratory by Mr. L. L. Bennett[1] that after chronic treatment with A. P. extracts, hypophysectomized rats become refractory to the action of these extracts in maintaining fasting muscle glycogen levels. In view of this finding, it seemed advisable to try chronic treatment in conjunction with the experiments described above.

A group of normal rats was therefore injected daily for 20 days with one cc. of the standard alkaline extract. They were then fasted 24 hours, fed glucose, and determinations of the disposition of the carbohydrate were made in the same manner as in the groups described in the previous paper. After this pre-treatment final injections of the A. P. extract, given 2 hours before the glucose was fed, had absolutely no effect on the respiratory quotients or on the deposition of the fed carbohydrate. Evidently, in these normal rats, after prolonged treatment with a beef anterior extract, a refractory state

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* Aided by the Presnell Fund for the study of the metabolic relations of the anterior hypophysis, contributed by Robert R. Presnell, Frank Tuttle, Arthur Stebbins, and Mrs. Gordon Kahn, of Los Angeles.

† Porter Fellow, 1937-38.

[1] Bennett, L. L., PROC. SOC. EXP. BIOL. AND MED. In press.

developed toward the anterior pituitary factor responsible for the depression in carbohydrate oxidation after glucose feeding. This effect would appear to be quite analogous to that obtained in fasting hypophysectomized rats on prolonged treatment with A. P. extracts.

9445

Inhibition of Somatic Growth in Castrate Rats with Pituitary Extracts.*

HENRY D. MOON. (Introduced by Herbert M. Evans.)

From the Institute of Experimental Biology, University of California, Berkeley.

Male and female rats were castrated when 22-23 days old, and intraperitoneal injections of adrenocorticotropic extracts[1] (A-C-T) were given daily beginning on the day following the operation. The growth of hair over the shaved operative areas and over the whole body was very greatly retarded in the animals given large amounts of A-C-T. There was retardation and almost complete inhibition of *somatic* growth when a high level of A-C-T was administered.

TABLE I.
Effect of A-C-T on Adrenal and Body Weights of Castrate Rats.

Rats	Wt. of Adrenals mg.	Total Dose of A-C-T units	Length of Treatment days	Initial Body Wt. gm.	Final Body Wt. gm.	Gain in Body Wt. gm.
3 ♂	60.0	13.2	16	49.9	107.0	57.1
3 ♂	28.0	Controls		51.3	132.0	80.7
2 ♀	50.5	13.0	14	51.5	99.0	47.5
3 ♀	24.5	Controls		51.3	123.3	72.0
3 ♀	107.9	42.0	28	48.3	96.0	47.7
5 ♀	47.8	Controls		48.0	155.2	107.2
2 ♀	152.2	42.2	23	45.0	65.5	20.5
3 ♀	35.7	Controls		47.8	138.3	90.5

There was a noticeable protrusion of the abdomen of the animals treated with the higher levels of A-C-T (42.0 + 42.2 units).[†] At

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* Aided by grants from the Board of Research, University of California, and the Rockefeller Foundation, New York City.

[1] Moon, H. D., PROC. SOC. EXP. BIOL. AND MED., 1937, **35**, 649.

[†] These animals are discussed with regard to their sexual development in the accompanying paper.

TABLE II.

Rats	Liver gm.	Spleen gm.	Kidney gm.	Digestive Tract gm.	Heart gm.	Thymus gm.	Two Thyroids mg.	Two Preputials mg.
2 castrate ♀ treated with 42.2 units A-C-T (body weight 65.5 gm.)	5.18	.55	.75	11.18	.37	completely atrophied	9.3	93.0
3 castrate ♀ controls (body weight 138.3 gm.)	6.40	.50	.64	15.26	.64	.47	26.5	45.0
3 normal ♀ 23 days old (body weight 48.6 gm.)	2.23	.26	.37	4.09	.33	—	—	—

autopsy the abdominal viscera of the injected animals appeared to be as large as those of the untreated spayed rats in spite of the disparity in body weight. The weights of the various organs were taken in the rats treated with 42.2 units of A-C-T, in the control spayed rats and in 23-day-old normal female rats.

There had been no inhibition in the growth of the spleen and kidney, and only a slight inhibition (?) in the growth of the liver and digestive tract of the treated rats. The heart and thyroids were considerably smaller than in the untreated spayed animals. The preputials were enlarged in the injected animals. The thymus was very atrophic and in some cases completely atrophied.

9446

Effect of Adrenocorticotropic Hormone on the Sexual Development of Spayed Rats.*

HENRY D. MOON. (Introduced by Herbert M. Evans.)

From Institute of Experimental Biology, University of California, Berkeley.

In a previous paper[1] the production of an androgenic substance by the adrenal cortex in castrate rats after the administration of adrenocorticotropic extracts (A-C-T) was reported. The production of an estrogenic substance by the adrenal cortex in spayed immature female rats after the administration of A-C-T is herein reported.†

Wyman,[2] Martin[3] and Corey and Britton[4] have demonstrated the cessation of estrus after adrenalectomy. Nice and Shiffer[5] were able to obtain premature sexual development in immature female rats with implants of rat adrenals. Frank[6] has reported an increase in

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* Aided by grants from the Board of Research, University of California, and the Rockefeller Foundation, New York City.

[1] Davidson, C. S., and Moon, H. D., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 281.

† Alcoholic extracts of steer adrenals have shown 1 intravaginal unit in 1-2 gm. of fresh tissue. The method of assay used was that of Lyons and Templeton (PROC. SOC. EXP. BIOL. AND MED., 1936, **33**, 587).

[2] Wyman, L. C., *Am. J. Physiol.*, 1928, **85**, 414.

[3] Martin, S. J., *Ibid.*, 1932, **100**, 180.

[4] Corey, E. L., and Britton, S. W., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 592.

[5] Nice, L. B., and Shiffer, A. L., *Endocrinol.*, 1931, **15**, 205.

[6] Frank, R. T., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 204.

the excretion of female sex hormone in patients with cortical tumors. Quantitatively negative results were reported by Corey and Britton[7] in ovariectomized rats and by Corey[8] in hypophysectomized rats in attempts to produce estrus with adrenal cortical extracts.

Immature female rats were spayed when 22-23 days old. Injections of A-C-T (prepared and assayed by the method previously published[9]) were given intraperitoneally beginning on the day following the operation. The vaginæ of the injected spayed rats opened after the administration of 30-42 units of A-C-T. The vaginæ of untreated spayed rats did not open.

TABLE I.

Rats	Opening of Vagina Days of Treatment	Daily Dose of A-C-T Units	Total Dose of A-C-T Units
W 43	24	1.5	36.0
W 45	24	1.5	36.0
BH 48	25	1.5	37.5
W 18	16	1.9	30.4
BH 28	18	1.9	34.2
B 05	22	1.9	41.8

Vaginal smears of the treated spayed rats showed the presence of cornified cells, a few nucleated epithelial cells and leucocytes. Animals W 18 and B 05 showed, 2 days after the opening of the vagina, vaginal smears consisting predominantly of leucocytes.

At autopsy the adrenals of the injected rats were found to be greatly hypertrophied.

9447 P

Effectiveness of Arachidonic Acid in Curing "Fat Deficiency" Disease.*

Osmo Turpeinen.† (Introduced by Herbert M. Evans.)

From the Institute of Experimental Biology, University of California.

The earlier work by Evans and Burr,[1] by Burr and collaborators,[2, 3] and by Evans and Lepkovsky[4] has definitely shown that the rat cannot maintain growth and well-being on diets rigidly devoid of fat, but invariably develops a characteristic deficiency disease. The vital need of the animal is not, however, as the same investigators have demonstrated, for fat in general but for certain unsaturated fatty acids. Of these, only linoleic and linolenic acids have so far proved effective in curing this specific deficiency, whereas oleic and alpha-eleostearic acids have been found inactive. Now, whether the 2 first-mentioned acids are the only ones capable of satisfying the need of the body for unsaturated fatty acids or whether other fatty acids with the same curative effect exist is naturally a question of great interest and of considerable theoretical significance.

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[7] Corey, E. L., and Britton, S. W., *Am. J. Physiol.*, 1934, **107**, 207.

[8] Corey, E. L., Proc. Soc. Exp. Biol. and Med., 1937, **36**, 41.

[9] Moon, H. D., Proc. Soc. Exp. Biol. and Med., 1937, **35**, 649.

* Aided by grants from the Board of Research and the College of Agriculture of the University of California, and the Rockefeller Foundation of New York City.

† Holder of a Finnish State Fellowship.

[1] Evans, H. M., and Burr, G. O., Proc. Soc. Exp. Biol. and Med., 1927, **24**, 740.

In the following, the results of some experiments with arachidonic acid are reported. This acid has been tested once before by Burr, Burr and Miller,[3] who found that an addition of methyl arachidonate as 10% to a mixture of equal parts of methyl linoleate and methyl linolenate slightly decreased the curative effect of the original mixture. This result did not, however, seem quite plausible, even to the above mentioned investigators, and it was felt by us that further tests with arachidonic acid were urgently needed.

Young female rats immediately after weaning were placed on a fat-low, casein-sucrose-salt diet supplemented with yeast and the non-saponifiable fractions from cod liver oil and wheat germ oil. When growth had ceased and the animals had reached a "plateau", they were deemed ready for tests. They were not used, however, before they had shown a constant or slightly declining weight over a period of at least 30 days. The test substances were fed together with the vitamin supplements daily (except Sundays). Arachidonic acid was given in the form of methyl arachidonate‡ at 2 levels, *viz.*, 33 and 100 mg. of the ester daily. The curative effect was measured mainly by the intensity of the renewed growth, for this was easiest to record quantitatively, although as a rule, close parallelism existed between the resumption of growth and the disappearance of other deficiency insignia (irregularity of ovulation, renal hemorrhage, etc.).

Results and Conclusions. The results are shown in the curves in

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[2] Burr, G. O., and Burr, M. M., *J. Biol. Chem.*, 1929, **82** 345; 1930, **86**, 587.

[3] Burr, G. O., Burr, M. M., and Miller, E. S., *J. Biol. Chem.*, 1932, **97**, 1.

[4] Evans, H. M., and Lepkovsky, S., *J. Biol. Chem.*, 1932, **96**, 143, 157.

‡ We wish to acknowledge the gift of this substance from Professor J. B. Brown of the Ohio State University, and Mr. George Y. Shinowara associated with him. Without it our work would have been impossible.

Fig. 1, each of which represents the average growth of a group of 3 rats. A curve demonstrating the growth response produced by 100 mg. of methyl linoleate daily is included for comparison. As the graph clearly indicates, growth is very similar in all 3 cases. The lag in the beginning of the 33 mg. curve is probably not significant because of the striking agreement of the curves in the later phases. These results leave no doubt as to the efficacy of arachidonic acid as a powerful curative agent in the "fat deficiency" disease. It is even more potent than linoleic acid. Some recent experiments, which will be described in detail elsewhere, have shown that about 100 mg. of methyl linoleate daily is needed to produce maximal growth in plateaued "fat-deficient" rats. The same effect can be obtained with 33 mg. of methyl arachidonic. Whether even smaller doses of arachidonic acid would give maximal growth response must be left undecided through lack of trials, but the tests reported here already justify the conclusion that arachidonic acid is at least approximately

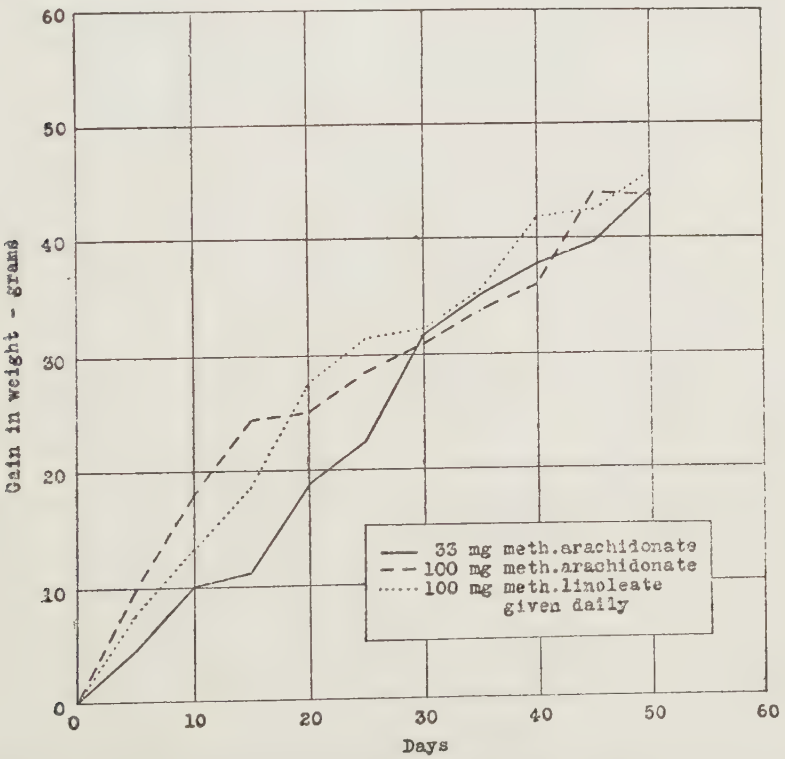

Fig. 1.

Growth Promoting Effects on Plateaued "Fat-Deficient" Female Rats of Methyl Arachidonate as Compared with Methyl Linoleate.

3 times as effective as linoleic acid in curing the "fat deficiency" disease.

The effectiveness of arachidonic acid as a curative agent might seem to be in contradiction to the finding by Spadola and Ellis[5] that the rat is able to synthesize this substance. The controversy can be eliminated, however, by assuming that this synthesis can take place only from (or in the presence of) linoleic (or linolenic) acid, which assumption seems to be in full accordance with the experimental data of the above mentioned workers. The superiority of arachidonic to linoleic acid as a curative substance would suggest the tentative hypothesis that the need of the animal organism might be primarily for the former acid and that linoleic (and linolenic) acid could be beneficial solely on account of its conversion into arachidonic acid in the body.

9448

Relation of Bile to Absorption of Vitamin E in the Rat.

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The present study is an extension of the previous work reported from this laboratory on the relation of bile to the absorption of the fat soluble vitamins.[1]

In order to determine whether or not vitamin E is absorbed by the bile fistula rat, female rats cannot be employed since female rats with bile fistulas fail to breed, and the usual criterion, *viz.*, the prevention of resorption of the fetuses by administration of vitamin E to the mother before the fifth day of pregnancy, cannot be used. Furthermore, if the bile fistula is established after breeding and before the eleventh day of pregnancy, resorption of the litter takes place.[2] Therefore the male rat was used in these experiments. In vitamin E deficiency, characteristic changes in the testes take place. The condition is irreversible. For this reason curative tests could not be used in the present study. The method of preventing the characteristic changes in the testes was used as the criterion for the absorption of vitamin E in bile fistula rats.

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[5] Spadola, J. M., and Ellis, N. R., *J. Biol. Chem.*, 1936, **113**, 205.

* E. R. Squibb and Sons Fellow. Technical assistance was supplied by the Works Progress Administration.

[1] Greaves, J. D., and Schmidt, C. L. A., *J. Biol. Chem.*, 1933, **102**, 101; *Univ. of Calif. Pub. Physiol.*, 1934, **8**, 43; *Am. J. Physiol.*, 1935, **111**, 492, 502; 1936, **116**, 456; PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 434.

[2] Unpublished work from this laboratory.

Three hundred fifty male rats, after weaning, were reared on a diet which was deficient in vitamin E and high in its content of fat.[3] At various intervals of time most of the animals were unilaterally castrated. The testis of each animal was weighed, sectioned, and studied histologically. Breeding tests were also carried out on these rats. Female rats of known fertility were employed in the breeding tests. In this manner the physiological state of the testis was correlated with the histological picture. The testis which was removed first served as a control. At the end of the experiment the second testis was removed and the histological pictures of the 2 were compared.

The weights of the first testes increased rapidly for a period of 50 to 70 days after the animals were placed on the diet. This was followed by a sudden and marked drop in the weights. This condition continued until about the hundred and twentieth day. The histological sections of these testes showed a progressive sloughing of the germ elements. In the earliest cases the condition manifested itself about the fortieth day and, in all cases, it was in evidence by the ninetieth day. There was a close parallelism between the weight of the testis and the degree of sloughing. The animals which did not receive supplements of vitamin E became sterile shortly after the hundredth day. The breeding data indicate that the animals reach sexual maturity about 30 to 60 days after being placed on the vitamin E free diets. After 60 days, increasing numbers of the animals gave histological evidence in the first testis of vitamin E deficiency. The condition was irreversible even though large amounts of vitamin E were administered. Most of the animals used in these experiments were castrated unilaterally shortly after the fortieth day, and only those which showed a normal picture on histological examination of the testis were used in the absorption studies.

A group of 82 animals served as controls. After unilateral castration they were placed on a low fat, vitamin E deficient diet.†

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[3] Hawk, P. B., and Bergeim, O., *Practical Physiological Chemistry*, 10th ed., p. 680.

† The diet consisted of alcohol-extracted casein 18%, cane sugar 69%, Osborne and Mendel salt mixture 4%, dried yeast 7%. A concentrate of vitamins A and D, in amounts equivalent to a level of 1% of cod liver oil, was injected into the bile fistula rats and added to the diet of the others. Methyl linolate from corn oil (0.4 cc. per week) was administered likewise.

Groups of these rats were subjected either to various degrees of inanition or to laparotomy. Another group received vitamin E. This was done in order to rule out the possible effect of these factors on the histological picture of the remaining testis. Administration of vitamin E markedly delays the sloughing process. However, once the process has been initiated, it is irreversible, and sterility ultimately results. The effect of inanition and laparotomy on the testis is rapidly and completely reversible.

The rest of the experimental animals were likewise castrated and placed on a vitamin E free low fat diet. Bile fistulas were established soon after the first testis was removed. These rats were then fed vitamin E concentrates, with or without deoxycholic acid, for extended periods of time. Twenty-five to 40 days after the bile fistulas were established many of the animals showed hemorrhages into the gastro-intestinal tract, the thoracic cavity, and even the cranial vault, At times the rats would bleed profusely as a result of minor injuries. The coagulation time of the blood was prolonged. By administering 1 to 2 cc. of beef bile orally the condition could be controlled for a period of about 10 days. It was then necessary to administer bile again. The procedure of administering vitamin E 4 to 5 times per week and a dose of bile in the interval was adopted in the absorption studies.

Comparison of the first and second testis of the bile fistula rats which received vitamin E‡ for periods of 2 to 6 months shows that the course of the testicular degeneration was delayed by the administration of vitamin E. This indicates that small amounts of this vitamin were absorbed. It is possible that the feeding of small quantities of bile weekly may have been responsible for the absorption of some vitamin E. A number of the animals which received vitamin E plus deoxycholic acid orally for periods as long as 420 days showed a normal histology of the testis, indicating that feeding deoxycholic acid definitely increases the availability of vitamin E in the bile fistula rat.

Summary. 1. Experiments have been carried out to determine the influence of deoxycholic acid on the absorption of vitamin E in the rat. 2. The prevention of degeneration in the testis was used as a criterion for the absorption of vitamin E. 3. The evidence indicates that administration of deoxycholic acid increases the availability of vitamin E, when given orally, in the bile fistula rat.

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‡ Each rat received 1 unit (Evans) of vitamin E in the form of the nonsaponifiable fraction of wheat germ oil 4 to 5 times weekly.

9449 P

Nature of the Factor Concerned in Loss of Blood Coagulability of Bile Fistula Rats.

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From the Division of Biochemistry, University of California Medical School, Berkeley.

In previous papers,[1] a marked increase in the coagulation time of the blood of the bile fistula rat was demonstrated. Analogous observations on dogs have been reported by Hawkins and Whipple.[2] The condition became evident when the animals had been maintained on a vitamin E free, low fat diet (See previous paper) or on the stock diet,† which was also low in fat, for a period of 30 to 50 days. The condition was not shown by unoperated rats when they were maintained on either of these diets. Small scratches or clipping of the tail in these bile fistula rats resulted in prolonged bleeding. At times this led to the death of the animal unless the bleeding was stopped by the application of collodion.

The content of prothrombin in the blood of the bile fistula rats decreased with time. The decrease after the prothrombin level had fallen to a certain point was roughly parallel to the hemorrhagic tendency. The method of Quick[3] was employed to estimate the prothrombin. Blood and brain tissues of normal rats were used to prepare the solutions. The normal serum as well as the serum which was treated with aluminum hydroxide were obtained from the pooled blood of a group of 8 to 10 normal rats. One-half cc. of blood was obtained by heart puncture from the bile fistula rats at intervals of 10 to 14 days. The prothrombin content of these animals was reduced to 20 to 30% of the normal before the coagulation time of the blood was markedly increased. Histological sections made from livers of some of the bile fistula rats when the blood coagulation time was markedly increased showed no evidence of liver damage.

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* E. R. Squibb and Sons Fellow. Technical assistance was supplied by the Works Progress Administration.

[1] Greaves, J. D., and Schmidt, C. L. A., *Am. J. Physiol.*, 1936, **116**, 456. Also accompanying paper.

[2] Hawkins, W. B., and Brinkhous, K. M., *J. Exp. Med.*, 1936, **63**, 795.

† The low fat stock diet contained 30 lbs. of wheat, 10 lbs. of whole milk powder, 1 lb. of alfalfa meal, and 100 gm. each of sodium chloride and calcium carbonate. The diet was supplemented with vitamin A and D.

[3] Quick, A. J., *Am. J. Physiol.*, 1937, **118**, 260.

All of the known dietary factors, such as vitamins A, D, E, B, and G, as well as the essential fatty acids, when added to the diet, had no effect on the hemorrhagic condition. Lemon juice, when fed at levels of 2 cc., likewise had no effect. On the other hand, when 2 to 3 cc. of beef bile were fed daily, a marked rise in the prothrombin content of the blood was noted within a period of 2 to 4 days after the bile was administered. The clotting time of the blood decreased. An ether extract of acidified bile was also effective. The activity of the factor was not destroyed by boiling the bile. Crude extracts of vitamin K (prepared from alfalfa), which had proven effective in preventing the hemorrhagic syndrome in chicks, as well as a highly purified preparation of vitamin K,‡ when fed to the bile fistula rat, were effective in decreasing the coagulation time of the blood and increasing the prothrombin level. The vitamin K preparations, after boiling with dilute alkali, were ineffective.

Similar loss in the coagulability of the blood with concurrent decrease in prothrombin was shown by rats which were maintained on the stock diet and were made icteric by ligation of the bile duct. When the flow of bile into the intestines was reëstablished in these animals, the prothrombin content of the blood increased to the normal level within a short time.

The factor which is essential in preventing the increased coagulation time and the decrease in the prothrombin content of the blood of the bile fistula and the icteric rat appears to be vitamin K or a substance which is closely associated with it. The question arises whether the absence of bile from the intestinal tract of the rat leads to failure in the absorption of the essential factor or whether the factor is excreted through the bile, or both. The low fat stock diet contained sufficient alfalfa meal to provide the vitamin K needs of the chick. Nevertheless, bile fistula rats developed loss in blood coagulability when maintained on this diet. Only when massive doses of vitamin K, without bile salts, or bile itself were fed did the coagulation time of the blood return to normal. These results appear to point to the possibility that bile acts as a carrying agent for the factor across the intestinal tract. Some absorption of the factor can, however, take place when massive doses of vitamin K are administered. Added evidence that certain of the bile salts favor utilization of the factor is gained from the fact that feeding crude bile salts, or bile which had been treated with alkali and heated, to bile fistula rats maintained on the low fat stock diet led to an increase in the prothrombin level and decrease in the coagulation time of the blood.

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‡ Kindly supplied by Dr. H. J. Almquist.

Further work on this problem is in progress.

Summary. 1. Bile fistula rats show loss in blood coagulability and a decrease in the prothrombin level. 2. This condition can be relieved by administration of a vitamin K concentrate.

9450

The Loss of Specific Substance in Washing Phase I *H. pertussis* Vaccines.*

JOHN J. MILLER, JR. (Introduced by Harold K. Faber.)

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Recent field studies of immunization against pertussis have yielded conflicting evidence. This is not altogether surprising in view of the fact that there is no standard method of preparing vaccines of *Hemophilus pertussis.* Recently isolated, toxic, Phase 1 strains[1] of *H. pertussis* have been used in the majority of recent trials.[2–5] Stock strains, however, are still used in preparing vaccine.[6] Although the newer knowledge of the antigenic changes of *H. pertussis* contraindicates the use of stock strains, it is obvious that a proposed immunizing agent must be judged only by careful clinical trial. Such trials as have been made with stock strain vaccines[7, 8] have not been sufficiently comprehensive to permit comparison.

Vaccines made from recently isolated, toxic, Phase 1 strains have differed principally in the amount of washing to which they were subjected. It is a current view[9] that the efficiency of any bacterial vaccine depends on (a) its production from virulent, smooth bacteria, and (b) the presence of the surface antigens of the virulent,

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* This work was aided by the Research Aid Fund of the National Research Council.

[1] Leslie, P. H., and Gardner, A. D., *J. Hygiene,* 1931, **31**, 423.

[2] Sauer, L., *Am. J. Pub. Health,* 1935, **25**, 1226.

[3] Kendrick, P., and Eldering, G., *Am. J. Pub. Health,* 1936, **26**, 8.

[4] Daughtry-Denmark, L., *Am. J. Dis. Child.,* 1936, **52**, 587.

[5] Doull, J. A., Shibley, G. S., and McClelland, J. E., *Am. J. Pub. Health,* 1936, **26**, 1097.

[6] Mishulow, L., Mowry, I., and Orange, R., *J. Pediat.,* 1936, **9**, 492.

[7] Park, W. H., *J. Pediat.,* 1935, **7**, 690.

[8] Shorr, E. Y., *J. Pediat.,* 1936, **9**, 49.

[9] Topley, W. W. C., and Wilson, G. S., *The Principles of Bacteriology and Immunity,* 2nd Edition, Baltimore, 1936, p. 831.

smooth bacteria. Furthermore, Lawson[10] has reported that Phase 1 strains have a capsule, demonstrable only when smeared directly without emulsification in an aqueous solution. The amount of washing to which an *H. pertussis* vaccine is subjected would therefore seem to influence its antigenic potency.

The following data were obtained when *H. pertussis* vaccine washings were tested for the presence of specific substance.

Rabbits were given intravenous injections of an unwashed suspension of Phase 1 *H. pertussis* in 0.85% NaCl containing 0.5% phenol. The vaccine contained approximately 11 billion organisms per cc. The strains used were less than one month old and were grown on Bordet-Gengou media containing either horse or human blood. Intravenous injections were given every 4 to 5 days, with occasional rest period, for a duration of 5 months. An initial dose of 0.1 cc. was gradually increased to 2.5 cc. Two weeks after the last injection the rabbits were bled. The serum was inactivated and stored unfiltered at 10° C. without the addition of any preservative. These sera agglutinated a recently isolated strain of *H. pertussis* to a titer of 1:12,000.

H. pertussis vaccine washings were prepared in the following manner. A slightly modified Bordet-Gengou medium[11] containing 33% goat blood was inoculated with 2 or 3 strains less than 3 weeks old (and less than 5 transplants old). The 2-day growth on 6 petri dishes was scraped off with a glass rod into 10 cc. of 0.85% solution of NaCl. The 2-day growth on the remaining 6 petri dishes was scraped into 10 cc. of distilled water. The suspensions were agitated 5 minutes to break up the clumps of bacteria and immediately centrifuged for one hour at 3000 r.p.m. The supernatant washwater was pipetted off, and then recentrifuged 5 to 9 times for one hour each time at 3000 r.p.m. After each centrifugation the washwater was transferred to another centrifuge tube and chilled before being replaced in the centrifuge. Centrifugation was carried out twice after all traces of sediment disappeared. (It may be mentioned that after the second hour of centrifugation the supernatant was uniformly water clear.) After this prolonged centrifugation smears and cultures of the washwater were negative. When run in duplicate, saline and distilled water washwaters were centrifuged for the same period of time.

The washwaters were tested for the presence of specific substance by a simple flocculation test. 0.2 cc. of washwater were mixed with 0.1 cc. of rabbit antiserum in varying dilutions. At first 0.85% NaCl was used as a diluent. Later 0.2% NaCl as suggested by Dean[12] was employed because of the greater rapidity of flocculation. To obtain a uniformity of salt concentration when titrating saline and distilled water washings in duplicate, sufficient NaCl was added to the distilled water washing to make the salt concentration 0.85% and then 0.85% NaCl was used as a diluent for both washing and antiserum. The tests were read at room temperature within 15 minutes and again after incubation for one hour at 37°C. The appearance of opalescent floccules was considered evidence of a union of specific substance (hapten or antigen) with antibody. Flocculation occurred when antisera in dilutions of 1-3, 1-9, and 1-27 were mixed with washwater in dilutions up to 1-8. (Tables I and II.) With undiluted antiserum flocculation was less constant and often slower, indicating antibody excess in the tubes. Four different lots of distilled water washings varied somewhat in the end dilution to which they were flocculated. Two lots of saline washings prepared simultaneously and under the same conditions as two lots of distilled water washings were flocculated in essentially the same dilutions as the distilled water washings. It would seem that

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[10] Lawson, G. M., *Am. J. Dis. Child.*, 1933, **46**, 1454.

[11] Kendrick, P., Miller, J. J., and Lawson, G. M., *Am. Pub. Health Assn. Year Book*, 1935-36, p. 200.

TABLE I.
Distilled Water Washings.

	Undiluted	1-2	1-4	1-8	1-16	Saline Control*
Antiserum	xx x –	xx x –	xx x –	xx x –	xx x –	xx x –
Undiluted	3 2 0	0 1 2	1 1 1	0 0 3	0 0 3	0 0 4
1-3	7 0 0	2 2 1	1 4 1	0 1 5	0 0 5	0 0 7
1-9	8 0 0	4 1 0	3 2 1	0 4 2	0 1 4	0 0 7
1-27	5 1 2	3 1 1	1 3 2	0 1 5	0 0 5	0 0 7
Saline Control*	0 0 8	0 0 5	0 0 6	0 0 6	0 0 5	0 0 7

Results of 9 titrations of distilled water washings. 4 lots tested against 2 antisera.

Figures indicate number of tests at indicated dilutions. Line demarcates positive zone.

xx = Flocculation occurring within 15 minutes at room temperature.
x = Flocculation appearing after incubation for 1 hour at 37°C.
– = No flocculation.

*In some titrations 0.85% NaCl was used as a diluent, in others 0.2% NaCl was used.

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[12] Dean, H. R., "A System of Bacteriology," *Medical Research Council, London,* 1931, **6**, 431.

saline and distilled water wash off about the same amount of specific substance.

TABLE II.
Saline (0.85% NaCl) Washings.

	Undiluted	1-2	1-4	1-8	1-16	Saline Control*
Antiserum	xx x –	xx x –	xx x –	xx x –	xx x –	xx x –
Undiluted	2 1 0	1 1 1	1 0 1	0 0 3	0 0 3	0 0 3
1-3	3 1 0	3 1 0	1 1 1	0 2 2	0 0 4	0 0 4
1-9	3 1 0	3 1 0	0 3 0	0 1 3	0 1 3	0 0 4
1-27	2 2 0	2 2 0	0 1 2	0 1 3	0 0 4	0 0 4
Saline Control*	0 0 4	0 0 4	0 0 3	0 0 4	0 0 4	0 0 4

Results of 5 titrations of saline (0.85% NaCl) washings. 2 lots tested against 2 antisera.
For legend see Table I.

As a positive control the dilutions of sera were titrated against an extract of *H. pertussis* prepared by freezing and thawing in distilled water.[13] Antiserum 1-81 and lower dilutions flocculated this extract immediately.

As other control flocculation tests showed the presence of serum (from the media) in the washwater it was necessary to test the rabbit antisera for the presence of anti-goat serum antibody. Traces of goat serum flocculating antibody were found in one of the antiserums when it was used undiluted, but not in a dilution of 1-3. Benzidin tests showed the presence of hemoglobin in the washwater. The rabbit sera were then titrated against wide dilutions of hemoglobin. No flocculation occurred. These negative control tests indicate the specificity of the flocculation reactions described above.

After the preparation of one lot of distilled water washings in the manner described, the sedimented bacterial cells of the primary centrifugation were then resuspended in an equal volume of distilled water. This was centrifuged for one hour at 3000 r.p.m. The supernatants constituting the 1st and 2nd washings were then recentrifuged until sterile. Flocculation tests were done. Antisera in dilutions of 1-9 and 1-27 flocculated a 1-8 dilution of the first washwater whereas they flocculated only a 1-2 dilution of the second washwater. This would indicate that the first brief washing removed considerably more specific substance than the second. Undoubtedly not all was removed by both washings.

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[13] Miller, J. J., *J. Immunol.*, 1934, **26**, 247.

A comparison was made of the agglutinability of *H. pertussis* bacterial cells before and after washing in distilled water. The cells were suspended in 0.85% NaCl and incubated for 20 hours at 37°C. Both unwashed and washed cells were agglutinated to a titer of 1-16,000. Apparently the washing in distilled water left sufficient surface antigen or hapten for combination with antibody.

It was found that Seitz filtration of the washwater removed all detectible specific substance. It has previously been noted that Berkefeld filtration of *H. pertussis* extracts removes the antigen which stimulates complement fixing antibodies in rabbits.[13]

The flocculation tests described above indicate that the Bordet-Gengou bacillus has a highly soluble surface which contains a specifically reacting substance. Whether this is a hapten or a complete antigen is not yet clear. Tests for the antigenicity of washings are in progress. It would seem certain, however, that a washed vaccine is not completely equipped with surface antigenic complex. On theoretical grounds, one must therefore agree with Sauer[14] in interdicting washing.

These findings are compatible with the favorable reports of Sauer[2] and Daughtry-Denmark[4] on the protective power of unwashed Phase I vaccine. They are also in line with the unfavorable report of Doull, Shibley and McClelland[5] on the use of a Phase 1 vaccine washed with distilled water. On the other hand these findings are less compatible with the evidence of Madsen[15] and Kendrick and Eldering[3] which indicates that a saline washed Phase 1 vaccine is an effective antigen. Apparently distilled water denatures the surface antigen in some way whereas saline simply removes a part of it. A comparison of the protective power in mice of saline washed and distilled water washed Phase 1 vaccine is being made.

Summary. Washing *H. pertussis* Phase 1 vaccine with either 0.85% NaCl or distilled water removes partially a specific substance which can be flocculated by hyperimmune rabbit serum. This finding constitutes grounds for omitting washing in the preparation of *H. pertussis* Phase 1 vaccine. It may explain in part the disparity in the reported results of immunization with Phase 1 vaccines.

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[14] Sauer, L. W., *J. A. M. A.*, 1934, **102**, 1471.

[15] Madsen, T., *J. A. M. A.*, 1933, **101**, 187.

9451 P

Hyperglycemia and Increased Liver Glycogen Values in Hypophysectomized Rats on Chronic Administration of Adrenocorticotropic Hormone.*

LESLIE L. BENNETT. (Introduced by Herbert M. Evans.)

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Previous work from this laboratory has shown that the injection of small amounts of suitable anterior lobe extracts into 24-hour fasted hypophysectomized rats prevents the abnormal fall of muscle glycogen without affecting the blood glucose or liver glycogen.[1] To produce this effect the extract is given only during the fasting period, and the presence of the adrenals is not necessary. Further investigation has shown that this acute effect is not due to adrenocorticotropic hormone.[2] The present investigation is concerned with the effects of more chronic injection of adrenocorticotropic hormone. The extracts were prepared according to the method of Lyons,[3] and all chemical and operative procedures were carried out as reported previously.[1]

TABLE I.
Carbohydrate Levels of Hypophysectomized Rats Fasted for 24 Hours after 10-24 Days' Treatment with BA-2 or L 191-4

	Muscle Glycogen mg.%		Liver Glycogen mg.%		Blood Glucose mg.%	
	Mean	Range	Mean	Range	Mean	Range
Untreated Normal (13)*	500	480-545	22	11-33	79	60-89
Untreated Hypophysectomized (8)*	298	253-389	22	18-26	52	42-61
Attempted Maintenance, 10 mg. BA-2 daily for 10-11 days (6)*	464	369-529	429	149-774	106	90-127
Attempted Repair, 10 mg. BA-2 daily for 11-12 days (5)*	488	438-592	86	20-212	92	72-108
Hypophysectomized-adrenal Demedullated Attempted Maintenance, 10-18 mg. BA-2 daily for 10-11 days (6)*	425	372-529	11	6-17	58	54-64
Attempted Maintenance, 5 mg. L 191-4 daily for 10 days (2)*	385	363-405	8	6-9	57	56-59

*Number of animals in the group.

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* Aided by the Presnell Fund for the study of the metabolic relations of the anterior hypophysis, contributed by Robert R. Presnell, Frank Tuttle, Arthur Stebbins, and Mrs. Gordon Kahn, of Los Angeles.

[1] Russell, J. A., and Bennett, L. L., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 406.

[2] Bennett, L. L., *Am. J. Physiol.* In press.

[3] Lyons, W. R., PROC. SOC. EXP. BIOL. AND MED., 1937, **35**, 645.

In Table I are summarized various experiments dealing with 10-24 days' injection of BA-2 (adrenocorticotropic hormone) and a purified lactogenic preparation. For the sake of convenience these experiments are called maintenance (injections started immediately after hypophysectomy) or repair (injections started 10-12 days after hypophysectomy). All extracts were injected 3 times during the fasting period in addition to the previous treatment. These data show that after longer treatment with adrenocorticotropic hormone there is a significant increase in liver glycogen and blood glucose. Not only are these values higher than those of the untreated hypophysectomized rat, but they are also far higher than those found in untreated normal rats. The adrenals of the maintenance group were much larger than those of the repair group, an average of 32.5 mg. as compared with 22 mg. These values should be compared with 25 mg. for untreated normal rats, and 10 mg. for untreated hypophysectomized rats. The only known contamination of BA-2 was one unit of lactogenic hormone per mg. The effects produced by BA-2 cannot be due to this contamination, as 50 units of lactogenic (5 times the amount administered in BA-2) were administered daily in L 191-4 (a potent lactogenic preparation supplied by Dr. W. R. Lyons) with clear-cut negative results.

The animals of one group were adrenal-demedullated about a week previous to hypophysectomy and after the latter operation maintained with BA-2. These animals grew normally during the interval between operations, and at autopsy serial sections of the adrenal remnants showed the presence of cortical tissue only. The blood glucose and liver glycogen values of these rats were not raised by treatment. This suggests that the effects are being mediated through the adrenal medulla. However, it is more likely that there was insufficient cortical tissue present to produce the elevation in blood glucose and liver glycogen. (In no case was the weight of the cortical remnant equal to the weight of the intact adrenals.) This interpretation is borne out by the observation of much higher carbohydrate levels in the maintenance than in the repair experiments. It is further supported by the fact that a large group of preliminary experiments, in which adrenocorticotropic extracts of varying potency were used, showed that a high degree of positive correlation existed between blood glucose level and the amount of adrenal repair.

These observations are in sharp contrast to the resistance which hypophysectomized rats develop to the metabolic effects of crude anterior lobe extracts after 10 to 20 days' treatment.[4]

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[4] Bennett, L. L., PROC. SOC. EXP. BIOL. AND MED. In press.

9452

Effect of Anterior Pituitary-Like Hormone on Lactation in the Albino Rat.

F. E. Connon.* (Introduced by Bennet M. Allen.)

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Experimental development of the mammary gland by administration of anterior pituitary-like hormone of pregnancy urine (A.P.L.) has been reported by several investigators. Bradbury[1] observed that the mammary glands of the mouse could be stimulated to develop by A.P.L. treatment, but only in those animals in which the uterus and ovaries were present. Selye, Collip, and Thomson[2] extended these findings to include the albino rat, and noted that increased development of the mammary glands paralleled an increase in the weight of the ovaries of A.P.L.-treated animals. No milk secretion occurred except after removal of the intensely luteinized ovaries, and then only in those animals in which the pituitary was intact.

In addition to a stimulative effect on the mammary gland, A.P.L. has been shown to inhibit milk secretion in normal lactating mice by Enzmann and Pincus,[3] and in normal and ovariectomized lactating mice by De Jongh.[4] Selye, Collip, and Thomson[5] administered A.P.L. and pig pituitary extract on the 3rd or 4th day after initiation of milk secretion in rats and observed an immediate inhibitory effect on lactation, which they suggest is exerted through the hypophysis rather than directly on the mammary glands. The mammary glands showed signs of secretion, but there was apparently lessened activity, since the suckling young of treated animals received little nourishment, lost weight, and eventually died.

The present work was undertaken to determine the effect of A.P.L. alone on lactation in the albino rat. Fifty-three lactating female animals of the Wistar strain and their litters were employed. Insofar as possible, litter-mate mothers were used and distributed among 3 groups: (1) normal untreated controls, (2) physiological saline-injected controls, and (3) Antuitrin S†-treated experimental animals. A standard number of 6 young per litter was maintained throughout by interchanging, when necessary, animals born on the same day. Intraperitoneal injection of the hormone was begun on the day of delivery of the young, since preliminary experiments had shown that there was little or no inhibitory effect on lactation in animals in which treatment was instituted several days after birth of litters. A.P.L. administration was continued from 3 to 5 days, the average daily injection being from 80 to 125 R.U. and the total amount given from 210 to 500 R.U. Injected controls were given physiological saline in volumes corresponding to the amounts of A.P.L. given, and over a period of time of 3 to 5 days. In preliminary work, 5 lactating animals were given from 20 to 50 R.U. per day, beginning on the day of delivery, and continuing for 3 to 5 days. No effect on lactation was noticed. The minimum effective daily injection was found to be about 80 R.U. Criteria for judging the inhibitory effect of the hormone were: (1) routine daily weighing of young and comparison of weight gains of control and experimental litters, (2) histological examination of mammary glands of the mothers in whole mounts and in sections as well as expression of milk through the nipples or cut edges of the glands after excision, and (3) examination of the stomach contents of the young animals. Mothers of control litters were sacrificed on the 21st day after delivery and those of experimental litters sacrificed on the 21st day or after death of the last young animal. Several mammary glands from each mother were fixed, and the ovaries and other endocrine organs removed for study.

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* The writer is grateful to Dr. Bennet M. Allen for helpful suggestions and criticisms throughout the course of this work.

[1] Bradbury, J. T., Proc. Soc. Exp. Biol. and Med., 1932, **30**, 212.

[2] Selye, H., Collip, J. B., and Thomson, D. L., *ibid.*, 1933, **30**, 588.

[3] Enzmann, E. V., and Pincus G., *Am. J. Physiol.*, 1933, **103**, 30.

[4] De Jongh, S. E., *Acta Brev. Neerl.*, 1933, **3**, 88.

[5] Selye, H., Collip, J. B., and Thomson, D. L., *Endocrinology*, 1934, **18**, 237.

† I am indebted to Dr. O. Kamm of Parke-Davis & Co. for a generous supply of Antuitrin S which was used in this experiment.

In the 24 cases where large doses of A.P.L. were given the young exhibited smaller daily weight gains than the controls, and in only 8 instances did some of the young of hormone treated mothers survive to 21 days. The total number of 6 young survived for the 3-week period in only 2 of the experimental litters. In every case, young of such litters died within a few days after the mother was sacrificed, whereas the young of control animals were capable of surviving after weaning in all cases. The average weight of the surviving young of experimental mothers on the 21st day was from 7.8 to 16.3 gm. as compared with the control average weight at 21 days of 23.2 gm. In the remaining 16 experimental litters, all young were dead from 2 to 16 days after birth, and averaged from 2.6 to 8.3 gm. at death or shortly before. In numerous instances, the young weighed less at death than the average of 5.2 gm. recorded at birth. The stomachs of the young animals were examined at autopsy. Those of animals from experimental litters were devoid of milk or contained only small amounts, while those of controls contained considerable milk in all individuals studied. The young of A.P.L. treated mothers continued to suckle until too weak to do so. The young of control litters survived the full period of 21 days, and after weaning, with the exception of 6 deaths in a total of 144 animals.

Microscopic examination of the mammary glands of the control mothers revealed full proliferation of all elements. Alveolar cells were vacuolated and exhibited evidence of active secretion. The alveoli were distended with milk. The nipples were fully developed and milk could be expressed from them and from the cut edges of glands excised for fixation. The mammary elements of the A.P.L.-treated animals were involuted in varying degrees, from complete breakdown of alveoli and terminal ducts to nearly full development of secretory structures. In the latter cases, secretion was dense and viscid and could not be expressed from the nipples which exhibited varying degrees of regression. Alveolar cells, when present in the more fully developed glands, where somewhat vacuolated and apparently secreting, but evidently not in normal fashion since the alveoli were not distended with milk. In general, the histological picture resembled that found in the normal involuting mammary gland and approximated to some extent the conditions described by Levenstein[6] in adrenalectomized lactating rats. Several mammary glands from each animal were studied to minimize the possibility of involution due to failure of the young to suckle. In each case, the glands from a single animal presented the same histological picture.

The ovaries of the experimental animals averaged 303.7 mg./kg. body weight as compared with 171.3 mg./kg. for the controls. Since there was some overlapping between the control and experimental ovary weights, the statistical significance of the data may be questioned. Histological study revealed intense luteinization in the ovaries of the A.P.L.-treated animals, this probably accounting for the increased weight. The high degree of luteinization suggested that the hormones of the corpus luteum possibly are involved in the inhibitory mechanism. However, this suggestion is not in accord with the observation of De Jongh,[4] who reported that A.P.L. inhibited lactation in ovariectomized mice. Also, a recent paper by Anselmino and Hoffmann[7] indicates that purified progesterone does not exert an inhibitory action on milk secretion, but they suggest that some other corpus luteum hormone may be concerned. It is possible that increased oestrone production accompanies the observable ovarian stimulation after A.P.L. treatment. Further work is required on this problem, and experiments are being undertaken in an attempt to clarify the situation.

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[6] Levenstein, I., *Anat. Rec.*, 1937, **67**, 477.

[7] Anselmino, K. J., and Hoffmann, F., *Zentralbl. f. Gynäk.*, 1936, **60**, 501.

Conclusions. While the mechanism of the inhibitory action of A.P.L. on lactation is not clear at present, it may be concluded that administration of large amounts of this hormone is effective in bringing about partial or complete cessation of milk secretion in the albino rat. The effect is measurable by routine weighing of the young and by histological examination of the mammary glands of the mothers. The results of this work confirm previous observations in which mice were employed as experimental animals.

9453 P

Excretion of Some Purgative Salts of Magnesium.

RICHARD J. WEBER. (Introduced by W. C. Hess.)

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There are few experimental studies recorded of the effect of ingestion of purgative salts of magnesium on the level of urinary and fecal magnesium. Hay[1] found that one normal man excreted through the kidneys in 23½ hours, 3.5% of the magnesium taken in a 20 gm. dose of magnesium sulphate. Yvon[2] found an increase of less than 5.0% in 24 hours in the urinary magnesium output after the ingestion of a similar dose of the same salt. Hirschfelder and Serles[3] reported that 7 normal men excreted from 40 to 44% (average 42.6%) of the magnesium taken in a single ordinary purgative dose of Epsom salt within 24 hours after ingestion. Haldane[4] recovered only 8% of the magnesium ingested in a 25 gm. dose of magnesium chloride.

Wry[5] recovered in one hour from the feces of a normal man 9.52 gm. or about 78% of the magnesium taken in a 12.25 gm. dose of magnesium sulphate; from the feces of another he recovered in one hour 12.5 gm. or 50% of the magnesium taken in a 25 gm. dose of the same salt. He observed that after 24 hours stools might still contain the purgative salt.

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[1] Hay, M., *J. Anat. and Physiol.*, 1882-83, **17**, 222.

[2] Yvon, M. P., *Arch. de Physiol., Normale et Path.*, 1898, **5**, 304.

[3] Hirschfelder, A. D., and Serles, E. R., *J. Clin. Invest.*, 1932, **11**, 841; *J. Biol. Chem.*, March, 1934.

[4] Haldane, J. B. S., *Biochem. J.*, 1925, **19**, 249.

[5] Wry, H., *Arch. für Verdauungs-Krankheiten*, 1909, **15**, 210.

It is apparent from these figures that there is considerable divergence in the results on the excretion of magnesium. It was considered, therefore, worthy of further study. The experiments were carried out on 8 human subjects whose ages ranged from 20 to 30 years. All subjects were in good health and were kept on a fairly uniform diet during the experimental period.

Magnesium and calcium determinations were made for 3 to 5 days prior to the experimental period on 24-hour collections of urine to determine the average daily magnesium and calcium excretion by this path. The calcium was determined according to the method of Kramer and Tisdall[6] and the magnesium by the method of Briggs.[7] Results by the above methods checked with gravimetric determinations of calcium and magnesium by the methods of McCrudden.[8] Two samples of urine gave 7.6 mg. and 5.1 mg. of Ca with the gravimetric and 7.3 mg. and 4.9 mg. of Ca with the volumetric determination; 6.2 mg. and 3.5 mg. of Mg with the gravimetric and 6.1 and 3.5 mg. of Mg with the colorimetric determination. The average daily urinary excretion of magnesium of the 8 individuals ranged from 83.9 to 132.1 mg.; of calcium from 141.1 to 364.9 mg. Five, 10, and 15 gm. doses of magnesium sulphate and doses of magnesium citrate containing an amount of magnesium equivalent to the amounts in the various doses of magnesium sulphate were taken orally. A period of at least one week was allowed to elapse between each ingestion, and with 2 exceptions the salts were taken before the morning meal. Two consecutive 24-hour samples of urine were collected in each case. The periods were measured from the time of intake.

In addition, the urine and feces were collected simultaneously for two 24-hour periods in 2 cases following the ingestion of 15 gm. of magnesium sulphate. Urinary calcium and magnesium were determined as above and analyses of the feces for the same elements were made according to the methods of Kramer and Tisdall.[6] Analyses of 24-hour samples of feces were made for several days prior to the ingestion of the salt to determine the normal magnesium and calcium content. The latter experiment was performed to determine whether the magnesium which did not appear in the urine could be accounted for in the feces.

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[6] Tisdall, F. F., and Kramer, B., *J. Biol. Chem.*, 1921, **48**, 1.

[7] Briggs, A. P., *J. Biol. Chem.*, 1924, **59**, 255.

[8] McCrudden, F. H., *J. Biol. Chem.*, 1909-10, **7**, 83; 1911-12, **10**, 187.

TABLE I.
Effect of Mg Ingestion on Urinary Mg.

Mg. of Mg as $MgSO_4$ Ingested	Urinary Mg mg.	%	mg.	mg.
1482	79.6	5.4	173.3	6.0
988	75.0	7.6	112.4	30.4
494	58.2	11.8	128.3	0.8
As Mg citrate				
1482	127.0	8.6	230.2	48.0
988	101.0	10.2	177.7	39.6
494	68.0	13.8	103.0	17.1
	average		range	

The data in Table I show that the oral intake of magnesium sulphate and magnesium citrate was followed by an increased excretion of magnesium in the urine. The amount of increase was directly proportional to the size of the dose and was greater following the administration of magnesium citrate. The percentage increase was inversely proportional to the size of the dose. In addition to the average results, the figures for maximum and minimum increase for each dose are given. The greatest percentage increase was 21.9% following the ingestion of 494 mg. of Mg as the sulphate. The intake of magnesium produced a variable effect on the level of urinary calcium (Table II). In most cases the level of urinary calcium was lowered, the decrease being greater following magnesium sulphate ingestion.

TABLE II.
Effect of Mg Ingestion on Urinary Ca.

Mg. of Mg Ingested	as Magnesium Sulphate Ca mg.	Ca %	as Magnesium Citrate Ca mg.	Ca %
1482	—24.8	—10.3	+12.3	+5.1
988	—35.1	—15.6	—27.7	—12.3
494	—30.0	—13.3	—8.9	—3.9
	average		average	

There was little or no retention of magnesium following the ingestion of a 15 gm. dose of magnesium sulphate by 2 subjects. Approximately 94% of the ingested magnesium appeared in the feces within 24 hours. In the case of the subject A, 100% of the ingested magnesium was recovered. Of this amount, 97.1% was recovered in the feces and 2.9% in the urine. In the case of subject B, 98.2% of the ingested magnesium was recovered. Of this amount, 90.5% was recovered in the feces and 7.7% in the urine. Subject A showed an increased calcium elimination of 735.1 mg. in the feces for the 48-hour period after ingestion and B showed an increased elimination of 1059.6 mg. for the same period.

9454 P

Occurrence of Organisms Resembling Pathogenic Members of the Colon-Typhoid Group in Long Stored Feces.

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When saline suspensions of human feces, stored in the icebox for many months, are plated on Endo's agar resultant colonial growth is different from that obtained with the same specimens when fresh. In most specimens, so stored and plated, there occurs an abundant growth of small, white colonies of Gram negative, non-sporing, generally motile, aerobic bacilli which exhibit a variety of biochemical activity, failing however to ferment lactose with acid and gas production, even after prolonged incubation.

Some of these organisms culturally resemble paratyphoid, typhoid and dysentery bacilli and may be mistaken for them. Furthermore, certain of the strains give serological cross-reactions with these known pathogens. Possibly they may be involved in gastro-enteritis, apparently water borne, since organisms similar to those we have under study have been reported from such outbreaks.[1]

Clemesha[2] was probably first to observe these organisms. For some reason he named them "Bacillus P", although noting they were a group of organisms not a species. Clemesha found them in fecal suspensions exposed in shallow dishes to the action of sunlight and observed that they appeared when the conditions of the experiment had eliminated the typical coliform bacteria of feces. Parr and Caldwell[3] encountered similar organisms in great numbers in water deriving from wells evidencing old fecal pollution as shown by chemical tests. Parr[4] again mentions them in a study on the viability of coliform bacteria and elsewhere he[5] states that they may be found occasionally in small numbers in fresh feces.

This report deals with 35 strains of the "Bacillus P" isolated from fecal suspensions stored in the icebox (4°C.) for a number of months and in a few instances isolated from fresh feces. The strains were purified by repeated plating and were then subjected to detailed pure-culture study. With 5 strains, examples of different cultural types, antisera were prepared.

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[1] Cooper, M. L., unpublished data.

[2] Clemesha, W. W., *The Bacteriology of Surface Waters in the Tropics,* Calcutta, 1912.

[3] Parr, L. W., and Caldwell, E. L., unpublished data.

[4] Parr, L. W., *J. Infect. Dis.,* 1937, **60**, 291.

[5] Parr, L. W., *J. Bacteriology,* 1937, **33**, 75.

Of the 35 strains 11 do not ferment any sugar; 18 strains produce acid, but no gas in dextrose-broth, and of these 7 also ferment lactose to the same degree. Six strains ferment dextrose producing both acid and gas. Two strains produce hydrogen sulphide and 6 liquefy gelatin. All strains but 4 are motile. Of the gelatin-liquefiers one is a typical *Proteus* but the other 5 are not. The "Imvic" reaction[6] (indol, methyl red, Voges-Proskauer, and citrate utilization) which is applied to coliform bacteria divides the 35 strains into 5 groups, *e. g.*, — — — +, — — — —, + + — —, — + — +, and — — + +, but these divisions do not correlate with fermentative differences.

Antisera prepared with 5 of these organisms were tested against 10 known strains, *e. g., E. typhi, S. sonnei, S. paradysenteriæ, S. paratyphi, S. schottmuelleri, S. enteritidis, S. choleræ suis, S. aertrycke,* Proteus X_2, and *A. fecalis.* No relationship was shown for Sonne dysentery but cross reactions occurred for *E. typhi* and *S. paradysenteriæ.* Several of our strains suggest *Alcaligenes fecalis* but the serum prepared with one gave only a weak reaction with a known *Alcaligenes* strain. Strongest cross-reactions were obtained with human types of *Salmonella* against serum No. 2 made with one of the paratyphoid-like organisms. Against a known *S. paratyphi* this serum effected agglutination when diluted 1/320, the titer against its engendering strain being 1/5120.

Such reactions mean no more than some degree of common antigenicity, but that cross-reactions as revealed in our preliminary studies do occur between known pathogens and bacteria existing in large numbers in long stored feces would seem to warrant further investigation of "Bacillus P."

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[6] Parr, L. W., *Am. J. Pub. Health,* 1936, **26**, 39.

9455

On the Alleged Oxytocic Activity of 1-Carnosine.

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Since the work of McClintock and Hines,[1] it has been accepted generally that carnosine, β-alanyl-*l*-histidine, possesses oxytocic properties. They reported that this dipeptide produced a marked increase in the tonal and rhythmical contractions of strips of guinea pig uterus when such strips were immersed in Tyrode's or Ringer's solution containing the compound in a 1:2000 dilution. They stated that this action was qualitatively and quantitatively similar to that produced by histamine in dilutions of 1:100,000. The carnosine was, therefore, regarded as having one-fiftieth the oxytocic power of histamine. They furthermore reported in this study that the material isolated from muscle tissue behaved identically in all their experiments to the synthetic carnosine prepared by Baumann and Ingvaldsen[2] which had been placed at their disposal.

With the synthesis of d-carnosine[3] the possibility of studying the relationship of stereostructure to the physiological activity of carnosine was afforded. As the first step in this comparison, duVigneaud and Hunt[3] studied the effect of both optical isomers on the blood pressure. The d-carnosine in 20 times the dose of l-carnosine was found to produce no perceptible decrease in the blood pressure of cats and rabbits under amytal and luminal anesthesia, thus demonstrating that the depressor action of carnosine depends upon the spatial configuration of the molecule.

In a continuation of this comparative study it was decided to test both isomers for oxytocic activity. Much to our surprise, however, when l-carnosine was tried on the isolated virgin guinea pig uterus no oxytocic action could be detected. It was likewise found that the d-isomer was inert in this respect.

The method employed in testing for oxytocic activity was that outlined in the United States Pharmacopoeia for the assay of posterior pituitary extracts. Histamine was used as a reference standard.

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[1] McClintock, J. T., and Hines, H. M., Proc. Soc. Exp. Biol. and Med., 1925, **22**, 515.

[2] Baumann, L., and Ingvaldsen, T., *J. Biol. Chem.*, 1918, **35**, 263.

[3] duVigneaud, V., and Hunt, M., *J. Biol. Chem.*, 1936, **115**, 93.

The concentrations of histamine which we considered effective, that is, capable of producing significant but submaximal contractions of the isolated uteri proved to be about one part per 10 million. This dose was one one-hundredth of that used by McClintock and Hines. The concentration they employed was far greater than that which we found necessary to invoke maximal contractions.

The l-carnosine used in these experiments was synthesized by the method of Sifferd and duVigneaud,[4] while the d-carnosine was prepared according to the directions of duVigneaud and Hunt.[3] The compounds possessed the physical constants reported in these papers.

Initially, we tried an amount of l-carnosine 50 times as great as the submaximal dose of histamine. This amount of carnosine, corresponding to a concentration of 1:300,000, proved to be without effect on the muscle strip. We then determined the least amount of histamine which would produce a perceptible contraction. This corresponded to a concentration of 1:150,000,000. We then found that carnosine in one thousand times this concentration was wholly without effect. In further efforts to determine whether we could get carnosine to stimulate the uterine strip under any conditions, we tested it repeatedly with different uterine strips in varying concentrations as high as 1:2000 without any perceptible effect on the tissue as far as an increase in tonus or rhythm was concerned. It was equally ineffective following various concentrations of histamine from 1:150,000,000 to as high as 1:100,000. It was also ineffective when posterior pituitary was used instead of histamine to establish the responsiveness of the tissue strips.

As a final step in this investigation, a highly purified sample of carnosine isolated from natural sources was tested for oxytocic activity. This sample had been isolated from fresh sheep muscle by a combination of the methods of Dietrich[5] and Gulewitsch and Amiradzibi[6] and is the sample utilized by Sifferd and duVigneaud in proving the identity of their synthetic product with naturally occurring carnosine.[4] No response was obtained with this sample in 2 trials at a concentration of 1:100,000, which corresponded in this experiment to one hundred times the submaximal dose of histamine.

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[4] Sifferd, R. H., and duVigneaud, V., *J. Biol. Chem.*, 1935, **108**, 753.

[5] Dietrich, M., *Z. physiol. Chem.*, 1914, **92**, 212.

[6] Gulewitsch, W., and Amiradzibi, S., *Ber. Deut. chem. Ges.*, 1900, **33**, 1902.

9456

Hyperglycemia in Response to Hypoglycemia in Normal and Hypophysectomized Dogs.

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Somogyi[1] has observed on diabetic as well as on non-diabetic human beings that hypoglycemia is followed by a compensatory hyperglycemia. After hypoglycemia in the postabsorptive state the blood sugar rises and in many cases temporarily exceeds the fasting level. In other words, there is a hyperglycemia relative to the fasting blood sugar. This phenomenon has not been generally noted because most workers do not observe the course of the blood sugar level long enough following hypoglycemia.

We report here the occurrence of the same phenomenon in normal and in hypophysectomized dogs, observed during studies of their

TABLE I.
Hyperglycemia Following Hypoglycemia in Dogs.
Figures represent mg. of glucose per 100 cc. blood.

Dog No.	Date	Time in Min. after administration glucose									Mg. % Increase
		0	30	60	90	120	180	240	300	360	
Normals											
105*	4/24/36	*81*	111	108	89	82	83	81	*87*		6
107*	2/11	*84*	116	99	82	*92*	84	80			8
108*	4/6	*75*	95	85	79	65	*81*	78			6
110*	3/30	*88*	103	98	88	*93*	92	94			5
A.1*	4/6	*87*	128	144	106	79	90	*94*			7
B.48†	7/16	*79*	66	*84*	83						5
06†	10/2	*77*	—	94	88	86	*107*	97	92	92	30
A.6†	7/16	*77*	121	64	—	*81*	76	78	79	75	4
B.38†	2/1/37	*76*	159	58	83	*87*	85	83	82	82	10
016†	2/16	*69*	114	72	73	71	*80*	73	74	73	11
08†	2/16	*76*	225	120	76	72	76	78	78	*81*	5
Hypophysectomized											
112*	12/31/35	*81*	114	124	101	77	86	*92*			11
A.1*	7/7/36	*79*	104	89	64	64	81	*85*	85		6
B.51*	7/7	*69*	84	83	73	68	71	*74*	69		5
06†	11/16	*60*	154	63	*76*	70	67	67	66	68	13
A.6†	11/16	*55*	114	55	56	60	61	63	63	*65*	10
B.1†	11/12	*53*	118	58	43	44	*63*	63	61	61	10
B.38†	4/5/37	*72*	366	256	151	101	66	64	73	*83*	11
016†	4/5	*66*	251	79	59	68	77	75	72	*77*	11
08†	3/15	*72*	199	49	55	77	73	77	74	*81*	9

*0.85 gm. of glucose per kg. body weight *per os.*
†1.50 gm. of glucose per kg. body weight intravenously.

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[1] Somogyi, M., unpublished.

carbohydrate metabolism. Hypoglycemia was produced by 2 different procedures: (1) by oral or intravenous administration of glucose; (2) by intravenous injection of moderate doses of insulin.

In the first type of experiment, several hours after the administration of glucose the blood sugar fell below the fasting level, and this hypoglycemia was followed by hyperglycemia within 6 hours after the administration. It may be seen in Table I that the compensatory hyperglycemic response after hypoglycemia occurred in hypophysectomized animals about as in the normals.

In the second group of experiments, hypoglycemia that did not reach the convulsive level was the result of the injection of insulin in moderate doses. As shown in Table II, the compensatory hyperglycemic response again appeared, as after the hypoglycemia elicited by the administration of glucose, both in normal and in hypophysectomized dogs.

TABLE II.
Hyperglycemia Following Hypoglycemia in Dogs.
Figures represent mg. of glucose per 100 cc. blood.

		Time in Min. after administration insulin									
Dog No.	Date	0	30	60	90	120	180	240	300	360	Mg. % Increase
Normals											
319*	6/17/36	*88*	64	62		93	*95*	93	92	95	7
A.4†	3/20	*79*	60	52		58	73	88	90	*93*	14
A.5†	2/21	*64*	50	56		64	75	80	*82*	82	18
B.51*	6/17	*68*	49	55		*72*	57	59	63	68	4
B.35*	6/17	*64*	67	60		60	70	*73*	70	73	9
06†	10/5	*68*	40	44		65	77	*82*	77	81	14
A.6†	10/5	*72*	43	39		58	71	73	74	*75*	3
B.75†	10/12	*88*	52	69		95	99	*101*	99	99	13
B.1†	10/12	*71*	40	35		50	67	*76*	75	73	5
105†	5/7	*66*	48	42		60	*89*	89			23
B.45†	10/5	*66*	42	35		65	*79*	77	76	79	13
05†	10/12	*80*	49	43		56	74	78	*91*	84	11
A.1†	4/7	*82*	68	49		59	73	*91*			9
B.38‡	1/29/37	*77*	64	43	52	63	83	82	*84*	84	7
016‡	1/29	*69*	67	31	37	46	63	82	*86*	84	17
08‡	1/29	*78*	47	54	66	83	83	*89*	80	82	9
Hypophysectomized											
A.4*	6/1/36	*74*	31	38		52	74	85	*88*	85	14
A.5*	6/25	*60*	43	49		61	73	68	*76*	76	16
B.35*	7/6	*63*	41	40		51	68	75	74	*78*	15
06*	11/12	*65*	45	32		48	58	71	*74*	74	9
A.6*	11/12	*65*	35	37		59	70	*76*	71	71	11
B.37*	6/1	*72*	38	31		36	58	82	82	*88*	15
B.38§	3/8/37	*52*	45	24	27	34	50	57	56	*61*	9
016§	3/8	*70*	29	22	33	37	63	69	*80*	79	10
08§	3/30	*71*	32	25	28	37	53	72	*83*	77	12

Dosages: * = 0.25 U/kg. body wt. all intravenous.
† = 0.5 U/kg.
‡ = 0.3 U/kg.
§ = 0.15 U/kg.

Instances of hyperglycemia in response to hypoglycemia are to be found in blood sugar curves after the injection of insulin, in experiments reported by Lucke, Heydeman and Hechler,[2] MacLeod,[3] and Scott and Dotti.[4] Scott and Dotti are the only ones to comment on it. With small doses of insulin, they state, "there may be a late hyperglycemia, occurring when the returning curve overshoots the original sugar level. This reminds one of the hypoglycemia so commonly seen in dextrose tolerance curves, though, of course, it is the reverse phenomenon."

Their remark is very much to the point. Whenever the glycemic level is changed from the normal fasting level, a compensatory change in the regulatory mechanism ensues to restore the original level. The remarkable constancy of the postabsorptive (fasting) level of glycemia is the result of a finely regulated balance between the glycogenolytic and glycogenetic processes.

When the blood sugar is raised to hyperglycemic levels, the compensatory hypoglycemia is usually attributed to the discharge of extra insulin as the result of a stimulus exerted by the hyperglycemia. The work of Soskin and Allweiss[5] seems to indicate that the hypoglycemic response may set in without extra insulin, as if hyperglycemia in itself could either depress glycogenolysis or intensify glycogenesis. This, of course, does not mean that insulin under physiological conditions has no part in the process; it is likely, however, that insulin is but one of the factors responsible for it. For the time being, it can only be said that upon the appearance of hyperglycemia a shift takes place in the balance between glycogenolytic and glycogenetic factors, causing the glycogenetic factors to outstrip the glycogenolytic ones. It is impossible at present to say whether this is the result of an increased activity of the former, or of a diminished activity of the latter, or whether both changes take place at the same time. The fact remains, however, that when a compensatory shift occurs in the relationship of the 2 factors, in many cases an overcompensation ensues, so that after hyperglycemia variable degrees of hypoglycemia are produced.

In contrast to hyperglycemia, hypoglycemia effects a shift in the balance between glycogenolytic and glycogenetic factors in such a manner as to cause the glycogenolytic activity to outstrip the glycogenetic. Here again the compensatory process frequently overshoots and produces a hyperglycemia in the sense that the blood sugar rises temporarily above the fasting level. This does not happen in all animals. In our experiments, the hypoglycemia which occurs following the administration of glucose elicited hyperglycemia in 11 out of 27 normal, and in 9 out of 21 hypophysectomized dogs. Insulin-hypoglycemia was followed by hyperglycemia in 16 out of 24 normal, and in 9 out of 21 hypophysectomized animals. The extent of the hyperglycemia shows considerable individual variations; in a few cases it represents a rise of no more than about 5 mg. % above the fasting level.

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[2] Lucke, H., Heydeman, E. R., and Hechler, R., *Z. f. d. ges. exp. Med.*, 1933, **87**, 103; **88**, 65.

[3] MacLeod, J. J. R., *Carbohydrate Metabolism and Insulin*, 1926, Longmans, Green and Company, Ltd., London.

[4] Scott, E. L., and Dotti, L. B., *Arch. Int. Med.*, 1932, **50**, 511.

[5] Soskin, S., and Allweiss, M. O., *Am. J. Physiol.*, 1934, **110**, 4.

The question may be raised as to whether such apparently trivial differences are sufficiently beyond the limits of analytical errors as to deserve consideration. The analytical technique employed in this work warrants an answer in the affirmative. True sugar values were determined by the copper-iodometric method with the Shaffer-Somogyi reagent No. 50[6] in filtrates prepared by Somogyi's copper precipitation method.[7] This procedure, in the hands of various workers in this laboratory, yields duplicate values which, as a rule, check within 1 mg. % ; the maximum discrepancy between duplicate determinations is 2 mg. %.

Thus if blood sugar values showed differences of more than 1 to 2 mg. %, these were safely beyond the limits of experimental errors and represented actual changes in the blood sugar level.

9457 P

Development of Autonomic Innervation Correlated with Reactivity of the Fetal Pig Iris.

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The results of recent studies of the responses of developing organs to certain choline esters have suggested an important rôle of the nerves in the initiation and progressive development of these reactions. Armstrong[1] has shown that when the embryonic Fundulus heart is differentiated to the adult morphology but is, as yet, without nerves, injections of large amounts of acetylcholine do not inhibit contractility. After the heart has become morphologically innervated, the injection of a smaller amount of the choline compound produces a definite vagomimetic effect, as shown at first by diastolic arrest of the auricle and later by diastolic arrest of both the auricle and the ventricle. Armstrong has suggested that acetylcholine probably produces its vagus effect by stimulating the vagus postganglionic fibers and that it is effective before the intramyocardial synapses are functional. In a study, the results of which have been reported, we[2] observed that instillation of carbaminoyl choline chloride on the eye of the fetal pig kept at room temperature elicited no constriction of the iris or only a slight constriction up to a certain stage of development. After that, the response became more marked for a certain period of development, until a maximum level of constriction was reached. It was suggested that probably a choline-labile receptive substance was beginning to form through contact of nerves with muscles in fetuses when little or no constriction was observed, and that this receptive substance might be one of several structures which develop gradually during the period of increase in the constriction observed. It had been planned[2] to determine by histological studies what relation the beginning of drug action has to the development of the parasympathetic nerves and the sphincter muscle; especially, what relation the response of the receptive substance to choline esters has to the differentiation of the nerve terminations.

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[6] Shaffer, P. A., and Somogyi, M., *J. Biol. Chem.*, 1933, **100**, 695.

[7] Somogyi, M., *J. Biol. Chem.*, 1931, **90**, 725.

[1] Armstrong, P. B., *J. Physiol.*, 1935, **84**, 20.

In most fetuses less than 100 mm. in length, no evidence of muscle histogenesis is to be observed. In fetuses just over 100 mm. in length, myoblasts of the sphincter muscle appear in the connective tissue at the pupillary margin of the iris just external to the pars iridica of the retina, where these myoblasts originate. In all fetuses 125 mm. in length a definite muscle mass has been formed. Differentiation progresses until in fetuses 175 to 200 mm. in length a thin but definite sphincter muscle is present in which the muscle cells appear completely formed. In fetuses 200 to 300 mm. in length growth in the individual muscle fibers and in the sphincter muscle, itself, is the most marked change.

Nerves are present early in the embryonic iris. Kuntz[3] has found that the ciliary ganglion, from which parasympathetic fibers to the iris have their origin, is well developed in pig embryos of 15 to 20 mm. and that many of its constituent cells have become differentiated into neuroblasts. The reactivity of the fetal iris to the carbaminoyl choline chloride[2] and the histological picture of the innervation of the iris in fetuses of the same length is indicated in the following summary.

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[2] Shaklee, A. O., Christensen, K., and Oppenheimer, H. E., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 225.

[3] Kuntz, A., *J. Comp. Neur.*, 1913, **23**, 71.

Fetuses 60 *to* 120 *mm. in length. In the younger individuals of this group, the iris usually does not constrict; in the older individuals it constricts only slightly* (*about* 3%). In the irises of fetuses 70 mm. or over in length which have been studied, the nerves are grouped at the periphery of the iris into bundles forming arcs which correspond to the major arterial circle. From these peripheral nerves, branches are given off radially which, especially in the younger fetuses, appear to accompany vessels toward the center of the iris. Smaller branches or individual fibers arising from these radial nerves reach the undifferentiated connective tissues where they form loose networks or accompany capillaries into the zone where histogenesis of the sphincter muscle occurs.

Fetuses 120 *to* 220 *mm. in length. In this group the constriction of the iris gradually increases from about* 3% *to the maximum obtained, about* 70%. The nerve development found in the smaller fetuses of this group has not advanced far beyond that observed in the preceding group which may be designated as the complete primordial development of the iris innervation. In the oldest fetuses of this group the innervation of the iris resembles that of the adult except as to quantity. In these fetuses simple branchings of slightly varicose fibers are found in contact with the sphincter muscle on its external border and scattered ramifying fibers appear within the muscle. Some of the fibers appear to end in beadlike terminations.

Fetuses 220 *to* 300 *mm. in length (to the end of the period of gestation). Although the percentage of constriction is lower in some fetuses,* 70% *represents the maximum constriction found for the fetuses of this group that were tested.* The branching fibers of nerves within the muscle become somewhat more abundant during the final stages of fetal differentiation. In particular, the nerve terminations become differentiated as in the adult with small loops or knoblike enlargements along their courses. The absence of a denser network of terminal endings in the late fetuses indicates that differentiation of terminal endings is not complete at birth. Many sphincter muscle fibers apparently have no direct contact with the terminal neural structure.

Summary. The microscopic study of the developing nerves of the iris indicates a very intimate relationship between the nerves and the differentiating sphincter muscle. The exact relationship between the initial innervation and the choline ester response has not been determined. More intimate morphological details of what occurs when the nerves make contact with the muscle fibers remain to be worked out. However, these findings suggest that the receptive substance may develop only after the nerves join the muscle fibers.

9458

Differential Survival in Isolated Strips of Frog Intestine.

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As part of the problem of survival of isolated tissue it became desirable to determine what elements within the tissue itself are responsible for the failure of spontaneous activity. Data are presented here correlating the physiological activity as measured by spontaneous movements with the histological picture in surviving strips of small intestine taken from the common leopard frog, *Rana pipiens* Shreber. In all, 50 frogs were used and over 250 series of activity records taken.

Segments one to 2 cm. in length, either intact or split lengthwise along the greatest curvature, were cut from the upper portion of the small intestine and transferred to shallow glass dishes containing 5 cc. of Ringer's fluid, buffered to pH 7.4-7.5 with carbonates and phosphates. No bacteriostatic substance was used, but the Ringer's fluid was changed twice daily. The strips were held at 5°C. except for an 80-minute period each day when each strip was mounted, by means of threads attached at the time of its removal from the frog, in a warm chamber filled with Ringer's fluid at 37.5°C. During this 80-minute period a continuous record of all spontaneous movements of the longitudinal muscle fibers was taken on an electric kymograph.

Frog intestine under such conditions survived for long periods as compared with the reported survivals of other smooth muscle preparations. One group of 16 frogs strictly comparable in all details shows the range of survival found in these tests, the minimal survival being 143 hours, the average 180 hours, the maximal 239 hours. Thirty-one percent of the survivals exceeded 200 hours. The maximal survival as indicated by the spontaneous activity of these frog intestine strips, 239 hours, was twice the survival time for activity of isolated rabbit intestine reported by Gunn and Underhill,[1] and equalled the maximal survival of irritability in the *retractor penis* of the horse, as demonstrated by Sertoli.[2] Isolated cat uterus given treatment similar to that accorded the frog intestine survived only 143 hours, a period equalled or exceeded by even the less hardy frog intestine strips.

The spontaneous movements of the longitudinal muscle were of 2 types, namely, large slow contractions of varying speed and amplitude upon which were superimposed small contractions of approximately uniform size and very regular rate. From 210 records of 26 frog strips these small regular contractions were found to occur at intervals of about 10 seconds when the excised frog strip was held at 37.5°C. The persistence of these small contractions throughout the entire survival period at approximately a 10-second rhythm and the regularity of this rhythm in all of the preparations of frog intestine studied were striking. Two exceptions must be noted, however, namely, that immediately after removal from the body of the frog, when the tissue was presumably in shock, and during the last run before spontaneous activity failed entirely, the rate of the small contractions was definitely slower, the rhythm being 12 to 15 seconds or longer.

Histological studies of surviving preparations taken from the activity series and fixed in Bouin's fluid at various times after removal from the frog were made throughout these experiments. As histological controls other strips were prepared as if for an activity series, and given the same daily routine treatment except that these control strips were held continuously at 5°C. and were never attached to the recording apparatus. One of these controls was carried for 478 hours. The final analyses showed that the experimental conditions imposed upon the strips of the activity series did not alter materially the sequence of the histological changes in these surviving strips as compared with the non-active controls, although these changes seemed to develop a little more slowly in the controls. The data from the activity series are presented.

During the first 2 or 3 days of survival very little change in the histological picture was observed, although the granules of the nuclei, particularly of the smooth muscle fibers, tended to become slightly coarser after 48 hours. At approximately 72 hours all muscle layers showed an increasing affinity for eosin, the maximal staining reaction with this dye being obtained between 72 and 100 hours.

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[1] Gunn, J. A., and Underhill, S. W. F., *Quart. J. Exp. Physiol.*, 1914, **8**, 275.

[2] Sertoli, E., *Arch. ital. de Biol.*, 1883, **3**, 78.

From 72 hours on the changes in the nuclei of the muscle cells were very evident. Each nucleus shrank, and wrinkled so that by the end of 150 hours of survival most of the muscle nuclei were definitely pyknotic and many were showing signs of fragmentation. After approximately 180 hours of isolation the muscle nuclei gradually lost their staining power, disintegrated, and disappeared from the preparation. By the end of 336 hours very little nuclear material remained in the muscle layers or throughout the strip, except a very definite line of pyknotic nuclei between the longitudinal and circular muscle layers, at the location of the ganglionic cells of the Auerbach's plexus.

A count of the nuclei per measured unit of cross-section of the longitudinal muscle layer showed that the disappearance of nuclei from the surviving strips could be divided into 3 phases. The first 240 hours of survival were marked by a steady but gradual decrease in the number of nuclei. Although during this period the muscle nuclei were undergoing definite degenerative changes, only about 5% of the nuclei actually disappeared per day. Near the 240-hour point a second phase developed lasting some 96 hours during which approximately 80% of the remaining nuclei disappeared and those nuclei which persisted became markedly altered, *i. e.,* this phase was characterized by rapid disappearance of nuclear material. From approximately the 336th hour of isolation to the final disintegration of the tissue the few nuclear remnants disappeared at the rate of about 1% of the original nuclear count per day.

As most of the surviving strips studied ceased spontaneous movements after 150 to 200 hours, and as the maximal survival was 239 hours, a correlation with the nuclear findings in the muscle cells suggests that the development of the pyknotic condition of the muscle cell nuclei which became general and widespread throughout the muscle layers between 150 and 200 hours was a determining factor in the cessation of spontaneous activity.

The survival of the cells of Auerbach's plexus in these isolated frog intestine strips was conspicuous. During the first 100 hours these ganglionic cells showed very little change, even their processes being well defined in properly fixed preparations. After 100 hours of survival the progress of nuclear change was much slower in the Auerbach cells than in the smooth muscle cells, the nuclei of these ganglionic cells being the last nuclei to disappear in the frog intestine strips. The persistence of the Auerbach cells in these surviving frog intestine strips is in accord with the findings of Cannon and Burket,[3] on the outstanding resistance of these cells in the cat intestine to pressure and low oxygen.

Since the cells of Auerbach in these surviving frog intestine strips showed little or no change until after the spontaneous activities ceased it seems probable that the Auerbach cells could have been functioning throughout the entire survival period of the muscle cells, *i. e.,* without additional evidence it would not be proper to consider the spontaneous movements of the surviving strips as entirely myogenic in origin in spite of the fact that the development of pyknosis in the muscle cell nuclei was correlated with the cessation of spontaneous movements.

9459

Loss of Potassium from Stimulated Frog Muscle.

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We have reported previously[1] that on stimulation rat muscles lose potassium in exchange for sodium, but that similar experiments with frog muscles showed no appreciable loss of potassium when the muscle was stimulated through its nerve. Other investigators[2, 3, 4] have had similar difficulty in demonstrating a loss of potassium with indirect stimulation of frog muscle. It now appears that a loss of potassium can readily be demonstrated in frog muscle if the nerve is stimulated with single shocks delivered at such a frequency that good contractions can be maintained for a period of 1.5 hours or perhaps less. If the muscle is continuously tetanized, the contractions fall off rapidly, not because of fatigue of the contractile mechanism but because of inhibition at the myoneural junction. Thus the potassium content, which seems to be related to the contractile mechanism rather than to the excitatory mechanism, is not affected.

The frogs *(Rana pipiens)* were usually anesthetized with 1 cc. of

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[3] Cannon, W. B., and Burket, I. R., *Am. J. Physiol.,* 1913, **32**, 347.

[1] Fenn, W. O., and Cobb, D. M., *Am. J. Physiol.,* 1936, **115**, 345.

[2] Mond, R., and Netter, H., *Arch. ges. Physiol.,* 1930, **224**, 702.

[3] Mitchell, P. H., and Wilson, J. W., *J. Gen. Physiol.,* 1921, **4**, 45.

[4] Ernst, E., and Csúcs, L., *Arch. ges. Physiol.,* 1929, **223**, 663.

20% urethane per 100 gm. body weight (otherwise etherized or decerebrated). The animal was fastened to a frog board, one Achilles tendon being cut and attached to an isotonic recording lever and the corresponding sciatic nerve being in contact with stimulating electrodes. After 1.5 hours of stimulation, both legs were clamped off, the gastrocnemius (and sometimes also tibialis plus peroneus) muscles removed, squeezed gently and blotted on filter paper to remove blood, and put into weighing bottles. Wet and dry weights (110°C. to constant weight) were obtained and the residue was transferred to platinum crucibles, ashed at 500°C. overnight, and analyzed for potassium. I am indebted to my assistant, Miss Doris M. Cobb, for the potassium analyses. The wet weights of the muscles varied from 0.59 to 1.15 gm. for the gastrocnemius, and from 0.27 to 0.57 gm. for the tibialis and peroneus.

TABLE I.
Stimulation of Frog Muscles at Varying Frequencies and Loads (per 100 gms. dry weight).

			Resting muscles			
Exp. No.	Load gm.	Frequency per min.	H_2O cc.	K millimols	ΔH_2O cc.	ΔK millimols
1	5	16-51	440	48.4	+94	—1.8
2	5	51	415	41.7	+57	—1.1
3	5	73	356	45.1	+90	—3.4
	5	73	352	46.1	t +55	—4.0
4	5	97	362	44.5	+76	—2.3
	5	97	356	45.9	t +59	—3.7
5	5	166	392	40.2	+117	—2.4
	5	166	381	40.4	t +111	—2.4
6	5	322	399	43.0	+101	—0.9
	5	322	412	41.7	t +108	—1.2
7	5	3420	411	46.4	+72	—1.8
	5	3420	416	47.1	t +99	—3.8
8†	57	93	384	49.2	+197	—4.2
9	57	93	348	45.5	+151	—3.0
10*	57	93	420	49.7	+127	—2.2
11*	57	93	396	50.0	t +84	—2.9
Average, 1.5 hr. intermittent			390	45.3	+100	—2.6
Average, 10-30 min. continuous (from Fenn and Cobb[1])					+121	—0.3

†Decerebrate.
*Ether anesthesia.
t = tibialis and peroneus muscles; all other gastrocnemius.
H_2O and K calculated per 100 gms. of dry weight of muscle. All animals under urethane except as otherwise noted. The load is the tension on the muscle itself.

The results are reported in Table I. In 16 pairs of muscles from 11 frogs, without exception, there was a loss of potassium and a gain of water. For comparison, the average loss in our previous series is included in the table. If contractions are maintained for a sufficient period of time, there is evidently no difficulty in demonstrating a typical loss of potassium in frog muscles, even with indirect stimulation.

At the higher frequencies listed in Table I, the contractions were continuous as in our earlier series but the loss of potassium was nevertheless evident. The greater loss observed in our present series as compared to the earlier series is not altogether due to the greater duration of stimulation, and maintenance of a muscle in a condition of "complete fatigue" by high frequency stimulation for 1.5 hours is not as effective in mobilizing potassium as intermittent contraction at lower frequencies. To show this we tried stimulating both legs of a frog at the same time, one leg at a low frequency so that it gave discrete twitches which were fairly constant in magnitude, and the other leg at a high frequency so that it gave a smooth tetanus which fell off rapidly in height. This frequency was usually 17/sec. but was varied somewhat in an effort to keep the contraction maximum. After 1.5 hours, the muscles were sampled and analyzed as before. The results (Table II) show a strikingly lower potassium content and a somewhat greater water content on the side where the contractions remained large, although the total number of stimuli delivered in the 1.5 hours was 17 times greater on the continuously stimulated side. The tension-time ($\int Tdt$) developed (above the initial tension of about 10 gm. in each muscle) was not accurately measured, but was certainly larger in the intermittently contracting muscle. Further experiments are necessary, however, to decide whether the loss of potassium is proportional to the performance of the muscle (tension-time) or to the degree of fatigue of the contractile mechanism proper.

TABLE II.
Continuous vs. Intermittent Stimulation in Frogs
(per 100 gms. dry weight).

	Water		Potassium	
	Continuous	Intermittent	Continuous	Intermittent
Exp. No.	cc.	cc.	m.-eq.	m.-eq.
1g	461	492	50.5	44.0
2g	537	568	50.0	45.0
2t	540	572	48.8	46.9

g = gastrocnemius; t = tibialis and peroneus. Frogs were under urethane. Contractions were recorded on a tension lever permitting considerable shortening. Initial tension about 10 gm. Duration of stimulation 1.5 hours.

There seems to be no direct conflict between these data and others previously published. Mitchell and Wilson[3] quote 2 experiments showing no loss of potassium due to indirect stimulation, but they used perfused muscles which might have already lost to the perfusate all the mobile potassium from both muscles of the pair, and they stimulated only for 2 seconds out of every minute with 30 minutes out of every hour for rest. Mond and Netter[2] and Ernst and Csucs[4] also used perfused muscles. The former authors stimulated for 30 minutes only, while the latter used short tetani every second until fatigued, repeating this several times, but do not mention the total duration of the stimulation. Ernst and Takács[5] quote 3 experiments showing a loss of potassium from perfused muscle indirectly stimulated but ascribe this result to the use of high potassium in the perfusate which was supposed to have increased the permeability of the muscle, thus permitting loss of potassium. Our results show that a high potassium concentration outside the muscle is not necessary for the phenomenon. We have not tried to introduce the unnecessary complication of perfusions in order to see whether a loss of potassium in activity can still be demonstrated by suitable stimulation under these conditions.

Summary. Frog muscles which are kept twitching rhythmically for 1.5 hours with well maintained contractions show a consistent average loss of 5.7% of the potassium originally present and a gain of water.

9460 P

Effect of Benzedrine Sulphate on Stomach Activity and Emptying Time.*

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Benzedrine sulphate is used clinically for the relief of pylorospasm and the facilitation of fluoroscopic and roentgenoscopic examination. Meyerson and Ritvo[1] reported that the drug brought about satisfactory disappearance of spastic manifestations in 85% of their cases.

On the other hand ephedrine sulphate, a closely related sympathomimetic compound, in doses of 65 mg. was reported by Van Liere, Lough, and Sleeth[2] as prolonging the emptying time of the stomach from 72.8 to 118%.

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[5] Ernst, E., and Takács, I., *Arch. ges. Physiol.*, 1931, **228**, 690.

* Aided by a grant from the Wisconsin Alumni Research Foundation.

[1] Meyerson and Ritvo, *J. A. M. A.*, 1936, **107**, 24.

[2] Van Liere, Lough, and Sleeth, *J. A. M. A.*, 1936, **106**, 535.

We were interested in determining the effect of benzedrine on initial and final emptying intervals and to what factors this action might be attributed.

Two series of fluoroscopic studies were carried out on normal dogs. Dogs in series A were selected for long initial opening intervals and were fed an opaque meal of 4.1 gm. of barium sulphate and 12.1 gm. of milk per kilo. The dogs weighing from 8.5 to 10 kilos were given 20 mg. of benzedrine orally; those from 10 to 14 kilos were given 30 mg. Dogs in series B were fed a thicker meal of 4.1 gm. barium sulphate, 5 gm. of powdered dog biscuits, and 12.1 gm. of milk per kilo of body weight. These were litter mates and weighed within 2 kilos of each other. They were all given 30 mg. doses of benzedrine, orally. In both series the interval between the time the drug was given and the opaque meal fed was from 10 to 15 minutes.

Table I summarizes the data concerning the effect of benzedrine sulphate on initial emptying of the stomach. In series A benzedrine reduced the average interval between the time the meal was given and its appearance in the intestine to 30.8% of the normal. The standard deviation of the normal initial emptying was ±3.4 minutes. The standard deviation of the initial interval following benzedrine was ±1 minute. In series B the average interval was reduced to 32% of the normal. In series B the standard deviation of the normal initial emptying was ±2.2 minutes, and the following benzedrine was ±1.2 minutes.

TABLE I.
Effect of Benzedrine Sulphate 20-30 mg. Orally, on the Initial Emptying Time of the Stomach. Series A and B.

	Normals		Benzedrine	
Dogs	Trials	Av. time	Trials	Av. time
A-1	3	13 min.	2	3 min.
A-2	5	10 ”	5	5 ”
A-3	4	15 ”	3	4 ”
A-4	5	14 ”	4	4 ”
B-1	3	15 ”	2	3 ”
B-2	3	14 ”	2	6 ”
B-3	3	12 ”	2	3 ”
B-4	3	14 ”	2	3.5 ”
B-5	3	11 ”	2	3 ”
B-6	3	10 ”	2	4 ”
B-7	3	11 ”	2	3.5 ”
Series A Average	17	13 ”	14	4 ”
Series B Average	21	12.5 ”	14	4 ”

From Table II it may be seen that benzedrine lengthened the final emptying periods by from 8 to 76 minutes, the average being 36 minutes.

TABLE II.

Effect of Benzedrine Sulphate, 20-30 mg. Orally, on Final Emptying Time of the Stomach. Series A.

	Normals			Benzedrine		
Dogs	Trials	Av. Time hr.	min.	Trials	Av. Time hr.	min.
A-1	4	3	39	2	4	55
A-2	4	4	12	3	4	44
A-3	4	4	29	5	4	37
A-4	4	5	17	3	5	27
A-5	5	4	33	3	5	47
A-6	3	3	53	4	4	14
Aver.	20	4	21	20	4	57

For the data in Table II the standard deviation of the difference of the averages is approximately ±20 minutes. The critical ratio is about 1.5, which would mean the data are probably not very significant. However, it must be noted that the average time of final emptying was increased for every one of 6 dogs under benzedrine. The chance that all would increase in this way is one in 64. The conclusion that benzedrine does cause a slight delay in final emptying seems, therefore, to be justified.

Records of the effect of benzedrine on stomach activity of dogs fasted for 12 to 18 hours were obtained by the balloon method. The balloon was connected through a water manometer to an air tambour so that a known pressure could be maintained in the balloon within the stomach. The drug, in solution (30 mg.), was introduced into the stomach through a second tube.

We find that the initial effect of the benzedrine occurs in about 8 minutes after it is given orally. The effect is one of increased tonus and rate of activity. Its secondary effect is one of marked inhibition of activity, even to cessation, which may or may not be accompanied by a slight loss of tonus. This latter effect takes place within 40 minutes.

We could demonstrate no constant effect of benzedrine on the intestine of the intact dog by manometrically recording the activity of the intestinal fistulæ. Water and mercury manometers were used. The benzedrine was administered orally, subcutaneously, intravenously, and by infusion into the fistulæ.

9461

Hydrogen ion Concentration and Anticoagulating and Fibrinolytic Action of Cultures of Streptococci and Pneumococci.

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Recently Neter and Witebsky[1, 2] have reported that many strains of *Streptococcus viridans* and pneumococcus were found to be fibrinolytic for human fibrin provided the organisms with which the tests were performed were grown in broth containing 2% dextrose. Tunnicliff[3] reported that strains of *Streptococcus viridans* were incapable of dissolving normal fibrin clot but that cultures of smooth forms inhibited coagulation. The latter author used for liquid culture medium meat extract broth containing one percent dextrose. Dennis and Berberian[4] reported that some strains of *Streptococcus hemolyticus* and *viridans* inhibited the formation of fibrin in plasma. They stated that 2% dextrose broth gave more active cultures than did 0.5% dextrose broth.

Because of the experience in this laboratory, using culture media containing 0.05% dextrose, that neither strains of *Streptococcus viridans* nor Pneumococcus have been encountered which were capable of dissolving human fibrin under the experimental conditions previously described,[5] additional observations have been made with special consideration being given to the dextrose content of the culture media in which the organisms were cultivated in relation to anticoagulating and fibrinolytic activity of strains of *Streptococcus hemolyticus, Streptococcus viridans,* and Pneumococcus.

Culture Media. Plain meat infusion broth containing one percent neo-peptone but no buffer, has been employed. Before use, the pH was adjusted to 7.4-7.6. Dextrose has been added to make concentration of 0.05%, 1.0%, and 2%.

Strains: Streptococcus hemolyticus. Ten strains derived from patients suffering from acute infections were employed. These strains were all known to be actively fibrinolytic.

Streptococcus viridans. Thirty-three strains, all of which were derived from patients with endocarditis, were tested.

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[1] Neter, E., and Witebsky, E., Proc. Soc. Exp. Biol. and Med., 1936, **34**, 549.

[2] Witebsky, E., and Neter, E., Proc. Soc. Exp. Biol. and Med., 1936, **34**, 858.

[3] Tunnicliff, R., *J. Inf. Dis.*, 1936, **58**, 92.

[4] Dennis, E. W., and Berberian, D., *J. Exp. Med.*, 1934, **60**, 581.

[5] Tillett, W. S., and Garner, R. L., *J. Exp. Med.*, 1933, **58**, 485.

Pneumococcus. Ten strains, all derived from patients with lobar pneumonia, were used.

All the cultures were approximately 18 to 24 hours old when used.

Test: The method was the same as previously described[5] and as used by the authors previously mentioned.[1-4] Immediately after $CaCl_2$ was added to the mixture of plasma and culture the tubes were placed in a waterbath at 37.5°C. and kept under continuous observation. The tubes were inspected at frequent intervals in order to note whether clot formation developed or was inhibited. Since the occurrence of fibrin dissolution necessitates the formation of fibrin, it is obvious that when coagulation failed to take place, the presence or absence of fibrinolysin could not be determined.

pH Determination: The broth cultures were centrifuged at high speed until the supernatant fluid was clear. 5-10 cc. were pipetted off and used in the tests for pH. LaMotte colorimetric block was used for most of the determinations. The range was from 4.4 to 8.4. Methyl red, chlorphenol red, and phenol red were used as indicators. In the experiment in which determination of pH in plasma-culture mixtures was made Beckman's glass electrode pH meter was employed.

The pH was determined on all cultures which were cultivated in broth containing 0.05%, 1.0%, and 2.0% dextrose. The tests with plasma concerning anticoagulating or fibrinolytic effect were carried out regularly at the same time with the whole broth culture; additional tests were also frequently made with the supernatant fluid of cultures which had been centrifuged.

The correlation between the pH of the full grown cultures and the result of tests for the prevention of coagulation or fibrinolytic action are presented in Table I.

An analysis of the table reveals the following: *Streptococcus viridans:* when grown in 0.05% dextrose broth the final pH of the cultures ranged from 6.6 to 7.2; coagulation occurred in every instance; dissolution of fibrin occurred in no instance.

When the same strains were grown in 1.0% dextrose broth the pH with 6 of the strains ranged between 5.0 and 5.6. With these 6 strains coagulation occurred, but no dissolution of fibrin. With the remaining 27 strains the acidity of the culture was found to range from pH 4.4 to 4.9. When tests were made with these cultures, coagulation did not occur. Since fibrin was not formed it is obvious that determination of fibrinolytic activity could not be made.

When the 33 strains of *Streptococcus viridans* were grown in 2% dextrose broth, the 8 cultures, which did not reduce the pH lower

TABLE I.

pH of Cultures in Relation to Coagulation and Fibrinolysis of Plasma.

% dextrose broth	*Strept. viridans*				Pneumococcus				*Strept. hemolyticus*			
	No. of strains	Range of pH	Coagulation*	Fibrinolysis†	No. of strains	Range of pH	Coagulation	Fibrinolysis	No. of strains	Range of pH	Coagulation	Fibrinolysis
0.05	33	6.6-7.2	33+	0	10	6.8-7.2	10+	0	10	6.6-7.2	10+	10
1.0	6	5.0-5.6	6+	0	6	5.1-5.6	6+	0	2	5.0-5.1	2+	2
									4	4.9	4+ Partial	4
	27	4.4-4.9	27—	X	4	4.8-5.0	4—	X	4	4.7-4.8	4—	X
2.0	8	5.1-6.0	8+	0	6	5.0-5.4	6+	0	4	5.1-5.2	4+	4
									4	4.9	4+ Partial	4
	25	4.4-4.8	25—	X	4	4.8	4—	X	2	4.8	2—	X

*Numerals refer to number of strains. + indicates clot formation. — indicates no clot formation.

†0 indicates no fibrinolysis occurred. X indicates no test since no clot formed. Numerals indicate number of strains causing fibrinolysis.

than 5, did not interfere with coagulation nor did fibrinolysis occur. The 25 strains which did form acid to the extent of lowering the pH below 5 inhibited coagulation.

The same intimate correlation between the pH of the test culture and the occurrence or inhibition of clot formation in the culture-plasma mixtures which occurred with the strains of *Streptococcus viridans* also held for the observations made with the 10 strains of Pneumococcus and 10 strains of *Streptococcus hemolyticus.* When the final pH was below 5.0, coagulation was prevented; when above 5.0, clotting occurred. *When coagulation occurred,* dissolution was brought about by the 10 strains of *Streptococcus hemolyticus* but the fibrin was unaffected by the 10 strains of Pneumococcus.

Additional evidence of the significance of the degree of acidity is brought out by tests for anticoagulating and fibrinolytic effect made with the following materials: (1) Sterile, uninoculated broth of varying pH; (2) Dextrose broth cultures with a final pH below 5, which were adjusted to above 5; (3) Plain broth cultures with a final pH above 5 which were adjusted to below 5. N/20 NaOH or N/1 acetic acid was used to adjust arbitrarily pH when necessary.

	pH 7.0		6.0		5.5		5.0		4.8-4.5	
	Coag.	Dis.	Coag.	Dis.	Coag.	Dis.	Coag.	Dis.	Coag.	Dis.
Sterile broth	Yes	No	Yes	No	Yes	No	Partial or No	No*	No	X
Strept. vir.	"	"	"	"	"	"	"	"	"	X
" *hem.*	"	Yes	"	Yes	"	Yes	"	Yes*	"	X
Pneumococcus	"	No	"	No	"	No	"	No*	"	X

*Result dependent upon whether or not partial coagulation occurred.

In the above experiment the formation of fibrin or inhibition of coagulation was directly related to the pH regardless of whether the degree of acidity was due to bacterial growth or was arbitrarily adjusted. Fibrinolysis occurred only with strains of *Streptococcus hemolyticus* at pH above 5. Below this degree of acidity the failure of coagulation to occur rendered impossible the determination of fibrin dissolution. When the dextrose broth culture of the strains of *Streptococcus viridans* and Pneumococcus, having pH below 5 and inhibiting coagulation were adjusted to pH above 5, coagulation occurred but no dissolution. When plain broth cultures of the same organisms, having pH usually 6.5 to 7.0 and not interfering with clot formation nor causing dissolution, were made acid to a degree of pH below 5, no clotting occurred and consequently lysis was not determinable. It may be further noted that sterile uninoculated broth, when adjusted to varying degrees of acidity, gave results indistinguishable from those obtained with cultures of *Streptococcus viridans* or Pneumococcus.

The final experiment consisted of a determination of the pH of plasma and cultures of *Streptococcus viridans,* the measurement being made after the materials were mixed. The results, correlated with the occurrence or absence of clot formation using the same materials, are contained in the following protocol:

Green strep. + plasma — pH of mixture 7.20 — clot 15 min.
" " + " — " " " 7.16 — " " "
" " + " — " " " 6.18 — " 30 "
" " + " — " " " 6.14 — " 60 "
" " + " — " " " 6.11 — " 45 "
" " + " — " " " 5.65 — no clot
" " + " — " " " 5.57 — " "

The results indicate the delayed effect on coagulation when the pH is 6.1 + or —, and the absence of coagulation at pH 5.5 to 5.6.

In a review of the physiology and pathology of blood coagulation, Wöhlisch[6] refers to the several investigators who have reported that pH 6.4-6.6 is optimal for fibrin formation. Eagle,[7] using fibrinogen and thrombin preparations, has found that fibrin formation is delayed at pH less than 6.2 and does not usually occur below 5.6. It is well known that streptococci and pneumococci produce a relatively high degree of acidity when grown in culture media containing appropriate amounts of sugar. It seems reasonable, therefore, to interpret the results which have been presented as indicating that the inhibition of coagulation by cultures grown in 1% or 2% dextrose broth is due not directly to a special property of the organism (antithrombin or antiprothrombin) which interferes with the phenomenon, but to the degree of acidity of the culture, which on addition to plasma, denatures the fibrinogen or other components involved in the process of blood coagulation. Recently Dennis and Adham[8] have reported results which emphasize the importance of acid contents of cultures in relation to anticlotting activity. The observations recorded in this article lend support to their findings.

It should also be emphasized that when the pH of any of the cultures was below 5.0, the failure of solid fibrin to form rendered impossible a determination of fibrinolytic activity. When the sugar content of the culture was so slight that growth of the organism did not lower the pH below 5.0 or when the acidity of the 1 or 2% dextrose broth cultures was arbitrarily altered to a pH sufficiently high to exclude the denaturing effect of acid, none of the strains of *Strep-*

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[6] Wöhlisch, E., *Ergebn. der Physiol.*, 1929, **28**, 443.

[7] Eagle, H., *J. Exp. Med.*, 1937, **65**, 613.

[8] Dennis, E. W., and Adham, L. D., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 84.

tococcus hemolyticus, viridans, or Pneumococcus inhibited coagulation; only the strains of *Streptococcus hemolyticus* induced fibrin dissolution.

9462

Bactericidal Action of Human Serum on Hemolytic Streptococci. Active Principle Obtained by Fractionation of Sera.

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The present report is part of an investigation concerning the capacity of serum from patients who are acutely ill to destroy hemolytic streptococci of the *beta* type. Previous reports[1] have demonstrated that the bactericidal property under consideration was demonstrable in the serum derived from patients during the period of active disease due to a variety of infections, but that following recovery from illness the streptococcidal activity was greatly diminished, as measured by the methods which were employed. Furthermore, normal sera have been found to be essentially devoid of streptococcidal activity and have served as controls throughout the observations.

The previous articles have described the technical procedures. In the present study a strain of *Streptococcus hemolyticus,* which has been found to be uniformly highly sensitive to the killing effect of patients' sera, has been used in all of the experiments. The samples of sera were derived from patients who were acutely, and usually severely ill from diseases such as pneumonia, or pyogenic infections due to different kinds of organisms.

Studies have been carried out in an attempt to isolate the active principle in serum which is responsible for the streptococcidal activity. Observations have been made with a protein-fraction and a non-protein constituent. The materials have been used separately and in combination.

The protein-fraction most regularly employed in the tests has been obtained by precipitation of serum at low temperatures with alcohol

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* John D. Archbold Fellow in Medicine, Department of Medicine.

[1] Tillett, W. S., *J. Exp. Med.*, 1937, **65**, 147, 163.

TABLE I.

Results of Streptococcidal Tests Performed with: A. Whole Serum; B. Protein-Fraction of Serum; C. Ultrafiltrate from Serum; D. Protein-Fraction Plus Ultrafiltrate.

Patient	No. 1		No. 2		No. 3		No. 4	
Test Material	6 hr.*	24 hr.*	6 hr.	24 hr.	6 hr.	24 hr.	6 hr.	24 hr.
P't serum (1 cc.)	†130	†—	250	7	2	—	11	—
P't Pr. (0.5 cc.) + Phys. S.S. (0.5 cc.)	† ∞	∞	∞	1000s	1000s	∞	320	∞
P't Ult. (1 cc.)	∞	600	∞	∞	∞	∞	∞	600
Norm. Ult. (1 cc.)	∞	1000s	∞	300	∞	∞		
P't Pr. (0.5 cc.) + P't Ult. (0.5 cc.)	46	1	400	1			26	—
P't Pr. (0.5 cc.) + Norm. Ult. (0.5 cc.)	160	6	500	4	23	—		
Normal	Normal		Normal		Normal		Normal	
Norm. Serum (1 cc.)	∞	∞	∞	∞	∞	∞	∞	∞
Norm. Pr. (0.5 cc.) + Phys. S.S.	∞	∞	∞	∞	1000s	∞	∞	∞
Norm. Pr. (0.5 cc.) + P't Ult. (0.5 cc.)	∞	1000s	∞	∞	∞	1000s	∞	∞
Norm. Pr. (0.5 cc.) + Norm. Ult. (0.5 cc.)	∞	∞	∞	280	∞	∞		

*Duration of incubation before subculture was made.

†Results of subculture. Numerals indicate number of colonies. — indicates no growth in subculture. 1000s and ∞ indicate innumerable colonies.

The number of colonies in subcultures taken at the beginning of the test was always 1000s to ∞.

according to the method described by Felton.[2] Sufficient 95% alcohol was added to serum to make a final concentration of 20%. The material was kept in the icebox overnight in order to obtain the maximal yield and was then spun down in a cold centrifuge. The supernatant fluid was drained as completely as possible from the packed sediment. The precipitate was redissolved in an amount of physiological salt solution equal to one-half the volume of the serum from which it was derived. The twofold concentration was, as the succeeding experiments will indicate, for the purpose of permitting the addition of non-protein constituents in equal quantity.

In determining the effect on hemolytic streptococci of the material obtained by alcoholic precipitation, 0.5 cc. of the protein solution was restored to the original volume of serum by the addition of 0.5 cc. of physiological salt solution or Ringer-Locke's solution and tested by the usual method for bactericidal action.

Data obtained from tests made with the protein fractions are contained in the table which also consolidates the results of experiments in which different materials were used separately and in combination. As the tabulated findings indicate, the alcoholic precipitate, when restored to volume with physiological salt solution and used in the tests, has not been found to possess the active principle of serum. The results obtained with the globulin derived from 4 separate specimens of serum are given in the table and are representative of other similar experiments.

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[2] Felton, L. D., *J. Immunol.*, 1931, **21**, 357.

In 2 instances, the first precipitation with alcohol yielded a fraction which possessed some killing power. However, following reprecipitation, negative results were obtained. It seems likely that in the first procedure, there was an incomplete separation of the constituents. The fraction derived from reprecipitation, although inactive alone, was effectively streptococcidal when mixed with ultrafiltrate according to the procedures to be described later in this article.

Attempts were made to obtain the non-protein constituents of serum by ultrafiltration through cellophane number 300 and sausage-casings. The apparatus used was that described by Lavietes.[3] Nitrogen-pressure equal to that of a column of mercury 30-35 cm. high was used to force the material through the membrane. The apparatus was placed in an incubator at 37.5°C. for 18 to 24 hours. At the end of this time 4 to 6 cc. of water-clear material had filtered from 10 cc. of serum. The sera were obtained from both patients and normal persons. Sterile precautions were used throughout.

It is interesting to note that with some of the samples of ultrafiltrate, although chemical tests for protein were negative, precipitative tests with anti-human rabbit sera were positive. This suggestive evidence of the occasional presence of small amounts of protein was detectable in the material filtered through cellophane but not in that passed through sausage-casing. It seems probable that different lots of cellophane differed in porosity. No difference was noted in the streptococcidal properties of the products of ultrafiltration regardless of the presence or absence of small amounts of protein. This minor variation in the constitution of ultrafiltrate has not seemed to be of significance in the present study.

The ultrafiltrate, water-clear at the beginning of the test, became turbid in approximately 3 hours after streptococci were introduced. At this time both stained smears and estimations of growth by diluted subcultures revealed marked increase in the number of organisms, which continued for 6 to 8 hours. At the end of 24 hours, innumerable viable organisms were present with some samples of ultrafiltrates, whereas with other samples considerable bacterial destruction had taken place. The variations just mentioned occurred with specimens of ultrafiltrate from both normal and patients' sera.

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[3] Lavietes, P. H., *J. Biol. Chem.*, 1937, **120**, 267.

In every test the initial increases in bacterial population were comparable. No explanation can be offered at the present time for the late bactericidal effect of some of the samples of ultrafiltrate. It should be noted, however, that the curve of rapid rise and fall of viable population which occurred in the occasional tests with ultrafiltrate was distinctly different from the progressive decrease in numbers of streptococci which have occurred in bactericidal tests with serum. It seems not unlikely, therefore, that the effect peculiar to some of the samples of ultrafiltrate may be dependent upon limitations in nutritional elements or some other undetermined factors rather than to the presence of an appreciable amount of the streptococcidal factor of whole serum. Further investigations are now in progress in an attempt to analyze the special effects of ultrafiltrate.

In addition to observations concerning the streptococcidal activity of the protein-fraction and the samples of ultrafiltrate used separately, tests have also been carried out with the 2 materials in combination. From the data presented in the table it may be seen that when the protein-fraction derived from patients' sera was combined with ultrafiltrate, the streptococcidal activity of the mixture was comparable to that of whole serum. It is interesting to note that in duplicating the bactericidal effect of serum, although the protein-element derived from patients' sera was a necessary constituent of the mixture, the ultrafiltrate from normal serum served equally as well as did the ultrafiltrate from patients' sera in restoring streptococcidal activity. The specific bactericidal principle seems, therefore, to be latently associated with the globulin of patients' sera obtained by precipitation with alcohol. In order to obtain bactericidal activity, however, the supplementing effect of a non-protein element, which could be derived from the serum of normal individuals as well as from that of patients, proved to be necessary.

That the bactericidal effect of the combined protein and ultrafiltrate operates through the same mechanism as that of serum, is further evidenced by the fact that the anaërobic conditions, which in previous studies were shown to inactivate the killing power of serum, also nullified the lethal action of protein-ultrafiltrate mixtures that were effective under aërobic conditions.

Additional comparative tests were made with serum and with the fractional mixtures with respect to the influence of hydrogen-ion concentration on the phenomenon. In a separate report,[4] results are presented which outline the ranges of pH in which the bactericidal action of serum is effective. The importance of hydrogen-ion concentration is also indicated by the fact that the serological property may be inactivated by reducing the pH below the appropriate levels. In the present experiments, the pH of all the products, whether derived from patients or normal individuals, was comparable at the beginning of the experiments. Consequently, the difference in the effect of patients' protein plus ultrafiltrate as contrasted with that obtained with normal protein plus ultrafiltrate is not primarily dependent upon the initial pH.

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[4] Tillett, W. S., and Stock, C. C., *J. Exp. Med.* In press.

A sample of ultrafiltrate was placed in boiling water and at intervals of 15, 30, 60 minutes, and at 2 hours, 0.5 cc. was removed, cooled, and added to 0.5 cc. of unheated protein-fraction derived from the serum of an acutely ill patient. The results were as follows:

	Subcultures	
	6 hr.	24 hr.
1. Patient's serum	210*	21
2. Pr. + Ult. unheated	250	10
3. Pr. + Ult. heated 15 min.	300	26
4. Pr. + Ult. " 30 "	400	120
5. Pr. + Ult. " 1 hr.	400	110
6. Pr. + Ult. " 2 "	∞	∞

*Number of colonies in subcultures.

The protocol indicates that the effect of heat was not significantly demonstrable until it had lasted for 2 hours. The observations just described have been repeated several times and in each instance the results have been essentially the same.

When the ultrafiltrate was heated, a faint but definite opalescence appeared after 15 to 20 minutes. The clouding did not however, appear to be associated with inactivation since the material was found after brief exposure to high temperature to retain the supplementing effect when added to protein.

In order to determine whether lipids were associated with the active principle present in ultrafiltrate, samples have been extracted with ether overnight and then tested in combination with a protein-fraction derived from patient's serum. The material treated with ether, plus protein, was found to be as potent as the original preparations.

When the preparations extracted with ether were heated, turbidity did not develop. It seems likely, therefore, that the clouding of untreated ultrafiltrate was due to the lipid constituent.

In all the experiments, in which fractions of serum have been tested separately and in combinations, material, composed of the protein-fraction from patient's serum plus ultrafiltrate has been found regularly to possess a marked degree of killing power. The constituents individually, however, have not, except for the variations previously discussed, given comparable results. The results suggest, therefore, that the active principle responsible for the destruction of streptococci consists of elements which are separable into inactive individual constituents. However, when mixed together, the streptococcidal property becomes effective. Studies at the present time are too incomplete to permit final interpretation of the results just described. Questions concerning the solubility of the protein preparation, or the influence of the concentration of electrolytes, or other possible factors associated with the complex chemical composition of serum require further investigation before the nature of the active principle can be completely defined. From the results so far obtained the significant constituent of ultrafiltrate seems to be neither protein nor ether-soluble lipid.

9463

Menstruation Inhibiting Action of Testosterone.

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The following experiments were performed to test the Androgen Testosterone Ciba for its possible replacement value of progesterone as an inhibitor of the menstrual flow.

1. *Inhibition of menstruation in the normal cycle.*

Monkey No. 245 was selected for this experiment for the great regularity of her menstrual cycles. These ran as follows: 1935-36: 28, 25, 24, 24, 24, 25, 28 days; 1936-37: 26, 25, 25, 26, 25, 26 days. The female was likewise ovulating with regularity and was fertile, a 56-day embryo having been removed by hysterotomy January 9, 1934.

Last menstrual period, March 23, followed by ovulation before April 13 (exact day not known).

Beginning April 13, 10 mg. of testosterone* was administered daily for 12 days, that is, to April 24 (except that 20 mg. was administered on Saturdays, none on Sundays; in this case 20 mg. was injected on the last day). Menstruation began on April 30. The succeeding cycle was 29 days in length.

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* The author wishes to thank the Ciba Company for the generous supply of Testosterone and Testosterone Proprionate placed at the disposal of the laboratory; likewise to E. R. Squibb and Sons for both material and the counsel of J. A. Morrell over a period of years.

Result: In this experiment the menstrual cycle was lengthened to 38 days as against an average of 25 or 26 days, a maximum of 28 days for the spontaneous cycles occurring previous to the experiment.

2. *Inhibition of menstruation after castration.*

Monkey No. 305. Received as mature menstruating female in September, 1934, weighing 4530 gm.

January 17, 1935, day 21 of cycle, ovarian follicle burst under palpating finger; corroborated by laparotomy January 18; menses, January 25; laparotomy January 28, ovary removed. It was found that no corpus luteum had formed.

December 2, 1935, day 8 of cycle, ovarian follicle again burst under palpating finger; laparotomy December 12, biopsy of corpus luteum and endometrium. Corpus luteum was found to be well developed.

December 18, 1936, female gave birth to normal male baby.

Menses occurred February 11, March 6, and March 30, 1937.

On April 15, day 17 of cycle, the remaining ovary was removed. From April 14 to 26 the animal received 10 mg. of free testosterone* daily as above. She was killed on April 27.

Result: The bleeding which is to be expected within less than 6 days after castration was in this experiment inhibited for 12 days, when the animal was killed.

3. *Inhibition of menstruation after estrin withdrawal.*

(a) Monkey No. 463. This multipara weighed 5540 gm. when she was received in August, 1936. She furnished a slightly pathological 15-day ovum excised by hysterotomy on October 16, when one ovary was also removed. A second but normal 15-day ovum was recovered on December 8 by the same means and the remaining ovary removed. The bleeding which followed the hysterotomy and castration stopped on February 16.

By injection of 100 R.U. daily of Amniotin Squibb* April 13 to 17, bleeding was effected April 22 to May 3.

The amniotin injection was repeated May 3-7, and from May 8 on 10 mg. Testosterone Proprionate Ciba* was injected daily as above until May 20, that is for 13 days. May 22, 15 days after discontinuance of estrin injection the animal was killed. No bleeding had taken place in this period.

(b) Monkey No. 330, a fertile female, furnished 2 embryos removed by hysterotomy. Castration was completed at the second operation on December 14, 1936.

In quite an unrelated experiment (mentioned here to complete the animal's history) a course of treatment with amniotin was given from January 18 to February 7, 1937, in gradually decreasing dosages and bleeding occurred February 8-17.

A second bleeding, as a control to the succeeding experiment, was effected May 8-11 by injection of 100 R.U. of amniotin daily from April 23-28. The injection was repeated for 5 days, May 17-21. The bleeding that would have occurred following this last injection was successfully inhibited for 63 days with testosterone proprionate injected as above (10 mg. daily). The last injection was made on the sixtieth day, the animal killed 3 days later.

Result: The menstrual bleeding which uniformly occurs in a favorable animal following injection of 500 R.U. of amniotin over a period of 5 days was inhibited for 63 days.

Incidentally it was observed that with the injection of testosterone the vaginal desquamation reaches zero, whereas with estrin it ranges up to 50% of the vaginal lavage. No pregravid transformation of the endometrium is brought about in the monkey (nor in the rabbit) by testosterone. On the other hand the mammary gland proliferates and grows acini in response to the male hormone testosterone and, surprisingly, the sex skin remains almost as red as with estrin injections (Amniotin Squibb or Progynon Sherring), although swelling was not observed with the dosage employed. The phallus was markedly enlarged.

Conclusion. Testosterone or Testosterone Proprionate inhibits the onset of menstruation in the normally cyclic monkey, after castration and after cessation of estrin injection.

In this sense this androgen simulates the action of progestin, as it does also in its stimulation of mammary development and in its inhibiting action on the vaginal mucosa. In its effect on the sex skin, however, it seems able to replace the estrogens, for this remained brilliantly red in a castrate for 63 days after estrin deprivation.

9464 P

Development of an Acquired Resistance to Bichloride of Mercury by Renal Epithelium in Proximal Convoluted Tubule.*

W. deB. MacNider.

From the University of North Carolina, Chapel Hill, N. C.

In previous publications[1-4] the fact has been established that when the epithelium of the convoluted tubules is sufficiently injured by uranium nitrate, a process of repair to this epithelial tissue may be inaugurated which results in the replacement of the damaged cuboidal cells by an atypical type of cell for this segment of the tubule. These cells are of a flattened order, imperfectly differentiated and with prominent, deeply staining nuclei. Such epithelial replacements not infrequently exist in the form of syncytial structures. An epithelial repair process of this type has been shown to impart to this segment of the tubule a resistance to subsequent injury by uranium nitrate. A fixed cell tissue resistance has been acquired in this segment of the tubule.

Some years ago the observation[5] was made during the study of the acute renal injury from bichloride of mercury that the predominant location in the kidney of the epithelial injury was the proximal convoluted tubule.

For several months 11 dogs have been available for study that have in past years effected a repair process to the proximal convoluted tubules as a result of a severe acute uranium injury which is characterized cytologically by the formation in this segment of the tubule of the atypical type of epithelial cell which has been previously described. All of these animals at different periods of their recovery have shown an acquired resistance on the part of this type of cell to secondary intoxications by uranium nitrate even when such intoxications were induced by an amount of this nephrotoxic agent 2 to 4 mg. per kilo in excess of the amount which induced an acute process of degeneration in the normal cuboidal cell of the proximal convoluted tubule. The object of the present experiments has been to ascertain whether or not the atypical regenerated epithelium which

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* This investigation was made possible through a grant from the Josiah Macy, Jr., Foundation.

[1] MacNider, Wm. deB., *J. Exp. Med.*, 1929, **49**, 387.

[2] MacNider, Wm. deB., *J. Exp. Med.*, 1929, **49**, 411.

[3] MacNider, Wm. deB., *Harvey Society Lectures*, 1928-29, Baltimore.

[4] MacNider, Wm. deB., *Science*, 1931, **73**, 103.

[5] MacNider, Wm. deB., *J. Exp. Med.*, 1918, **27**, 519.

has repaired the convoluted tubule segment and which has shown resistance to uranium is also resistant to a poison acting rather specifically on the same segment of the nephron: bichloride of mercury.

The 11 dogs used in these experiments were given subcutaneously at the time of their use 0.25 cc. of a 4% solution of morphine sulfate per kilo in order to induce emesis and later a certain degree of narcosis which state would favor the retention of a solution of bichloride of mercury. This poison was then given by stomach tube in solution in the amount of 15 mg. per kilo. The appearance of mercury in the urine was determined by employing a delicate test devised by Elliott.[6] The test is of some quantitative value. Two of the 11 dogs died in a state of shock due to an intense gastro-enteritis within 48 hours following the commencement of the intoxication. Mercury was present in the urine of both animals. The atypical epithelium in the convoluted tubule segment showed no histological evidence of injury. The 9 remaining animals developed a gastro-enteritis which varied in intensity and duration. They recovered from it. The urine of these dogs indicated the presence of mercury over periods varying from 2 to 8 days for the different experiments. The urine which contained both albumin and casts failed to undergo any marked reduction in the 24-hour total output. Biopsy material from the kidneys of these animals obtained during the periods of intoxication which was stained for histological study by Herxheimer's Scharlach R method for lipoid material and eosin and methylene blue for general study, has shown glomeruli without evidence of acute degenerative changes and in the proximal convoluted segment of the tubule, lined by atypical cells an epithelium in which both nuclei and cytoplasm stained normally and which failed to show the presence of stainable lipoid material. Such cells have failed to participate in the severe edema and vacuolation followed by necrosis which is characteristic of the epithelial injury from bichloride of mercury. The inference appears permissible, that an atypical type of epithelial cell which developed in the convoluted tubule segment of the nephron as a result of a repair process from an acute uranium intoxication and which later showed an acquired resistance to this poison has also acquired a resistance to bichloride of mercury which exerts its predominant toxic action on the epithelium of this segment of the nephron.

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[6] Elliott, J. A., *J. Am. Med. Assn.*, 1917, **68**, 1693.

9465 P

Cerebrospinal Fluid Studies in Convulsive Disorders.

Mona Spiegel-Adolf.

From the Department of Colloid Chemistry, D. J. McCarthy Foundation, Temple University School of Medicine, Philadelphia.

Absence of pathological findings in cerebrospinal fluids of epileptics is considered the rule (Lennox and Merritt[1]). Nevertheless it seems probable that any process involving chronic destructive changes of the brain tissue should cause the appearance of protein and lipoid cleavage products in the cerebrospinal fluid. In order to detect such cleavage products interferometry and conductivity methods have been used. The NaCl concentrations which give the same conductivity as the cerebrospinal fluids are computed and expressed in interferometric values (= IE, the interferometric values of the electrolytes). When IE is deducted from the interferometric value of the cerebrospinal fluid, the difference is equal to the interferometric value of the nonelectrolytes (= INE). This is represented in the cerebrospinal fluid by proteins, sugar, lipoids, cleavage products. The ratio INE/IE is independent of concentration changes in the cerebrospinal fluid. Furthermore, by combining the results of the physicochemical methods with the results of chemical investigations (protein, sugar, chlorides determinations) it is possible to compute the residual interferometric value (RIV), which is not accounted for by the said determinations. This is done by computing the protein and sugar concentrations in terms of interferometric values and deducting these values from INE.

In Table I the average INE/IE and RIV found in 60 cases with convulsive seizures are recorded together with the analogous values found in normal cases (14), in cases with other organic nervous disturbances (49), and in cases with brain tumors (25).

A comparison of the figures indicates that the average INE/IE and

TABLE I.
The Nonelectrolyte-electrolyte Ratio (INE/IE) in 60 Cases of Convulsive Seizure.

	INE/IE	RIV
Normal cases (14), (Hysteria, Headache, Surgical cases)	.22	53
Organic nerv. diseases (49), (Convulsions and brain tumors excluded)	.30	82
Convulsive disorders (60)	.32	92
Brain tumors (25)	.69	109

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[1] Lennox, W. G., and Merritt, H. H., *J. Neurol. and Psychopath.*, 1936, **17**, 97.

RIV in convulsive states is not only higher than in the normal cases, but also higher than in the cases with other nervous disorders and inferior only to the analogous figures in brain tumors. This seems to indicate that products not covered by the usual chemical investigations, *e. g.*, lipoids and other brain cleavage products, exist in increased amounts in the cerebrospinal fluids of cases with convulsive seizures. These conclusions are partially confirmed by recent reports of lipoid increase in the cerebrospinal fluid of epileptics.[2]

A closer examination of the individual cases with convulsive disorders shows that there seems to exist a relationship between the severity of the case and the frequency of the seizures on the one hand, and the INE/IE and the RIV on the other hand. Thirty-five cases with grand mal attacks were classified as severe either because of long duration, increasing frequency of attacks or subsequent mental deterioration. These cases showed the highest INE/IE and RIV. The lowest corresponding values were shown by 8 cases of symptomatic convulsions (allergy, calcified brain tumor, hypoglycemia, metabolic or endocrine disorders, depressed skull fracture, etc.). Mild cases of "genuine" convulsions (10 cases), showing only petit mal attacks or marked improvement for several years, and all babies with convulsions (3 cases) have INE/IE and RIV between the severe genuine and the symptomatic cases. In 4 cases the available clinical information did not permit a classification. The results are summarized in Table II.

TABLE II.
60 Cases of Convulsive Disorders.

Classification	No. of cases	INE/IE	RIV
Severe cases of grand mal: *a.* Lasting for several years *b.* Getting worse *c.* Mental deterioration	35	.34	97
Mild cases *a.* Showing only petit mal *b.* Showing improvement	10	.30	86
Babies (up to 23 months) with convulsions	3	.27	86
Symptomatic convulsions	8	.27	68
Clinical diagnosis not clear	4	.32	94

These results seem to point to a possible diagnostic and prognostic value of the INE/IE and RIV determinations in convulsive disorders.

The author expresses her thanks to Dr. T. Fay and Dr. W. Babcock for supplying the cerebrospinal fluids.

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[2] Knauer, H., and Heidrich, L., *Z. f. d. ges. Neurol.*, 1931, **136**, 433.

9466 P

Rhythmic Activity of Single Nerve Fibers Induced by Low Calcium.

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In the course of certain experiments on the chemical excitation of nerve we have observed the development of rhythmic volleys of impulses in the individual fibers which recur at regular intervals for long periods of time. Because of the possible relation of such a phenomenon to the mechanism of rhythmic activity of the nervous system in normal and pathological states we have studied this type of response in some detail.

All of the experiments have been performed on the sciatic nerve of the frog. In order to facilitate the penetration of the solution used for stimulation the sheath of the excised nerve was removed for a length of about one cm. and here the fibers were carefully separated so as to form a large number of small bundles. The region thus treated with the solution was usually at one end, but it was equally satisfactory to do so anywhere along the course of the nerve. A single fiber was then isolated at the other end of the nerve and placed on non-polarizable electrodes leading to an amplifier and oscillograph.

Partial removal of the ionized calcium in the nerve by sodium citrate or a reduction of its concentration by bathing the fiber bundles in calcium-free Ringer's fluid initiates a continuous train of impulses from the treated end at the rate of about 100 per sec. When the nerve fiber has been brought into this condition which causes a sustained discharge of impulses we find that the substitution of normal Ringer's fluid for the citrate or the calcium-free solution ultimately transforms the discharge into a series of rhythmic volleys. The continuous discharge goes on for a time which is variable from preparation to preparation and then suddenly stops, followed after an interval of a few seconds by a burst of impulses. The activity of the nerve has now become rhythmic for succeeding volleys of impulses are discharged from the treated region.

Both the durations of the volleys and the intervals between successive volleys undergo progressive changes as the normal physicochemical conditions of the nerve are gradually restored. There is a continuous decline in the duration of the active period—or the volley—while the intervals at first increase in duration and then decrease. After this period of transition a fairly constant state is attained which may continue for 30 min. or more, and during this time the durations of the successive volleys and intervals remain remarkably constant. Ultimately the intervals between the volleys grow longer again, and the resting condition of the nerve is reached as the interval goes to infinity.

During the period of regular and rhythmic activity the duration of the volleys may be several seconds and the intervals 10 or 15 sec., or, as has frequently occurred, each volley may continue for 1 or 2 min., and recur every 10 min. But even at these longer intervals the volleys and the periods of silence are constant to within a few percent for an hour or more.

The impulses within a volley follow a definite sequence. Whereas the original spontaneous activity consists of a train of impulses of fairly constant frequency the impulses within a burst start at a high frequency of 300 to 350 per sec., drop off to about 100 per sec., and then cease abruptly.

The character of this activity resembles in certain respects that of injured mammalian nerve fibers reported by Adrian[1] and alcohol treated crustacean fibers studied by Fessard.[2] The phenomena which we are reporting are, however, initiated by physiologically significant factors and are completely reversible so that the whole process can be stopped at will and the nerve returned to a resting condition by applying a solution containing an excess of ionized calcium. The value of such a controllable preparation of peripheral nerve tissue for the study of the fundamental mechanisms of rhythmicity will be obvious. It is not unlikely that the basis of such mechanisms and of our observations will be found in chemically induced alterations of irritability described by Lehmann[3] and by Katz.[4]

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[1] Adrian, E. D., *Proc. Roy Soc. (B)*, 1930, **106**, 596.

[2] Fessard, A., *L'Activité Rythmique des Nerfs Isoles*, Paris, 1936.

[3] Lehmann, J. E., *Am. J. Physiol.*, 1937, **118**, 613.

[4] Katz, B., *J. Physiol.*, 1936, **88**, 239.

9467

Cobalt Polycythemia in the Dog.

JOHN EMERSON DAVIS. (Introduced by H. B. Haag.)

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The hematopoietic power of cobalt was first demonstrated on the rat by Waltner.[1] Orten and coworkers[2, 3, 4] have shown that a daily dose of 0.5 mg. cobalt, with copper and iron, produces in rats a true polycythemia involving an increase of the blood volume, and that cobalt probably acts by stimulating the hematopoietic organs. Kleinberg[5] reported that the subcutaneous injection of cobalt chloride produces in rabbits a polycythemia, and an increased reticulocyte percentage. Mascherpa[6] reported that the oral administration of cobalt to dogs produces an increased erythrocyte number. Brewer,[7] however, reported that the daily feeding of cobalt chloride in doses of 0.8 to 4 mg. of cobalt per kg. failed to produce a significant polycythemia in his dogs. He asserts that the erythrocyte number in normal dogs may vary by 15 to 20% as observed on consecutive days.

In view of these conflicting reports on the effect of cobalt upon the erythrocyte number of the dog, I decided to reinvestigate the question.

Four dogs used in this investigation were fed a uniform daily diet of Purina dog chow. Two of these dogs were normal females, while the other 2 (males) were polycythemic due to the fact that they had finished a prolonged period of daily treadmill running just 3 weeks before the cobalt feeding was commenced.

Cobalt was administered in the form of cobaltous chloride in 1:1000 solution (cobalt equivalent) by stomach tube each day just before feeding. It was found that the salt could not be given in gelatin capsules, as this form of administration caused the dogs to vomit. During the first period of cobalt feeding, 2 of the dogs were given 2 mg. cobalt per kg. daily, while the other 2 were given

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[1] Waltner, K., *Arch. Exp. Path. u. Pharm.*, 1929, **141**, 123.

[2] Orten, J. M., Underhill, F. A., Mugrage, E. R., and Lewis, R. C., *J. Biol. Chem.*, 1932, **96**, 11.

[3] Orten, J. M., *J. Biol. Chem.*, 1933, **99**, 457.

[4] Orten, J. M., *Am. J. Physiol.*, 1936, **114**, 414.

[5] Kleinberg, W., *Am. J. Physiol.*, 1934, **108**, 545.

[6] Mascherpa, P., *Arch. ital. de Biol.*, 1930, **82**, 112.

[7] Brewer, G., *Am. J. Physiol.*, 1937, **118**, 207.

TABLE I.

Effect of Cobalt Feeding on Erythrocyte Number and Hemoglobin Percentage in Dogs.

Physically Trained Dogs						Normal Dogs					
Dog 1			Dog 2			Dog (6)			Dog (7)		
Date	R.B.C.s Millions	Hb Sahli %	Date	R.B.C.s Millions	Hb Sahli %	Date	R.B.C.s Millions	Hb Sahli %	Date	R.B.C.s Millions	Hb Sahli %
3/24	4.76	68	3/24	5.76	87	3/27	5.02	71	3/19	5.00	72
3/25	4.76	68	3/27	5.77	85	3/31	5.14	73	3/22	5.39	72
3/30	4.66	71	3/30	5.83	89	4/6	5.12	68	3/29	5.15	68
4/6	4.74	70	4/1	5.81	86	4/9	5.19	72	4/2	5.10	68
4/14	4.90	69	4/4	5.68	82	5/3	5.25	71	4/29	5.32	71
4/16	5.21	69	4/16	Exercise discontinued		5/4	5.26	71			
4/16	Exercise discontinued		5/3	5.93	78						
4/30	4.96	69	5/4	5.93	78						
Cobaltous Chloride Administration (Commenced 5/5)											
2 mg. Cobalt per kilo daily			2 mg. Cobalt per kilo daily			6 mg. Cobalt per kilo per day					
5/8	5.71	74	5/8	6.18	82	5/7	4.87	69	5/8	5.75	69
5/11	5.28	72	5/10	6.13	79	5/11	5.29	72	5/10	5.55	68
5/14	4.83	70	5/13	5.88	78	5/13	5.29	76	5/14	6.06	73
5/17	4.91	71	5/15	6.24	80	5/15	6.08	74	5/17	6.30	69
5/19	5.14	73	5/18	5.67	81	5/19	6.58	76	5/19	5.82	71
5/21	5.14	71	5/21	5.68	76	5/21	6.51	79	5/22	5.93	73
5/24	4.88	69	5/24	5.56	78	5/24	6.24	79	5/24	5.55	70
5/25	4.96	68	5/24	Cobalt discontinued		5/29	6.16	81	5/29	Cobalt discontinued	
5/27	Cobalt discontinued		5/31	5.02	76	5/29	Cobalt discontinued		6/1	5.50	70
5/31	4.81	68	6/4	5.06	74	6/1	5.72	80	6/4	5.32	67
6/4	4.64	70	6/8	4.82	69	6/4	4.86	81	6/8	4.42	67
6/8	4.36	70	6/12	4.69	69	6/8	4.85	81	6/11	4.75	70
6/12	4.12	68	6/15	4.63	68	6/11	5.03	80	6/14	4.81	72
6/15	4.19	70				6/14	4.90	80			
6/16	Cobalt Administration: 2 mg. per kilo daily										
6/19	4.90	74	6/22	5.29	71	6/17	4.97	80	6/18	4.77	71
6/24	5.20	73	6/24	5.60	74	6/22	5.74	81	6/22	5.10	71
6/28	5.07	73	6/28	5.82	75	6/25	5.75	83	6/24	5.25	71
6/30	4.99	71	6/29	5.72	74	6/29	6.05	83	6/28	5.75	72
7/2	5.06	72	7/1	5.65	73	7/1	6.03	82	6/29	5.99	73
									6/30	5.83	74

6 mg. After 3 weeks the cobalt was discontinued for about 3 weeks, then all 4 dogs were given a uniform dose of 2 mg. cobalt per kg. daily over a period of about 2 weeks.

Red blood cell counts and hemoglobin percentages (Sahli) were determined frequently throughout the control and experimental periods. Blood was drawn from the saphenous vein while the animal was lying quietly (blindfolded) upon a table and was in a basal state with respect to previous exercise and food consumption.

Before the first period of cobalt feeding dogs 1 and 2 had erythrocyte numbers which were already 20% and 15% higher than their pre-exercise normal values. These chronic exercise polycythemias are a confirmation of previous work by the author.[5]

It will be noted in Table I that the high erythrocyte numbers of dogs 1 and 2 were maintained throughout the first 3-week period of cobalt feeding and, altogether, for 6 weeks after the cessation of daily exercise. Within 2 weeks after the discontinuation of cobalt, the cell counts dropped by 16 to 18%. On 6 mg. cobalt per kg. daily, normal dogs 6 and 7 developed significant polycythemias within 10 days. The increase in the erythrocyte numbers amounted to about 18%. Within 10 days of cessation of cobalt administration the cell counts returned to normal.

On June 16th, a second experiment (Table I) was started in which each of the 4 dogs was given 2 mg. cobalt per kg. per day. Within 4 to 12 days, the erythrocyte numbers of all 4 dogs had increased by about 20%. This increase is of approximately the same order as that observed by Mascherpa[6] in a cobalt-fed dog, and by Davis and Brewer[8] in physically trained dogs. Probably, we cannot expect to induce a large (50%) increase of erythrocytes in the dog by these methods.

My results in this and previous work force me to disagree with Brewer[7] on the range of day to day variations in the erythrocyte number of the normal dog. To obtain fairly consistent counts, it is important that blood be drawn only when the animal is quiet and unexcited. Otherwise the red cell counts will be too high, perhaps signifying an emotional polycythemia in the dog. In any event, it is known that the spleen contracts during excitement, and forces additional blood cells out into the circulation (Barcroft[9]). When all proper precautions are taken, the normal erythrocyte number of the dog should not vary by more than 7% as determined on consecutive days. This is probably a very generous allowance for the range of variability.

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[8] Davis, J. E., and Brewer, N., *Am. J. Physiol.*, 1935, **113**, 586.

[9] Barcroft, J., and Stephens, J., *J. Physiol.*, 1927, **64**, 1.

Throughout these experiments, the animals appeared to be in excellent condition. They did not lose any weight, and showed *no* anorexia or lethargy. Body temperatures remained quite normal.

Conclusions. The oral administration of 2 mg. cobalt (as cobalt chloride) per kg. daily to dogs produces a significant increase (about 20%) in the erythrocyte number. No toxic symptoms were observed in dogs which were fed as much as 6 mg. cobalt per kg. daily for 3 weeks.

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Comparative Study of Fibrinolytic and Anticoagulating Properties of *Streptococcus hemolyticus* and *Streptococcus fecalis* (Enterococcus).

ERWIN NETER AND ERNEST WITEBSKY.*

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Tillett and Garner[1] made the interesting observation that hemolytic streptococci may produce a powerful fibrinolysin. Their findings were corroborated and extended by several authors, including Dart, Dennis and Berberian, Van Deventer, Hare and Colebrook, Hatfield, Magee and Perry, Lippard, Johnson and Wheeler, Madison, Meyers, Keefer and Holmes, Reich, Schmidt, Stuart-Harris, Tunnicliff and others. The fibrinolysin produced by hemolytic streptococci *in vitro* has the following main properties: It dissolves human but not animal plasma-clots, with the exception of *rhesus* plasma, as demonstrated by Van Deventer and Reich[2]; it is produced in infusion-broth in rather high concentration, and it may be neutralized by streptococcal antiserum as well as by serums of patients recovering from streptococcal infections. Besides the fibrinolysin, glucose-broth cultures of hemolytic streptococci may contain a second factor which inhibits coagulation of both human and animal plasma, as observed

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* Aided by a grant of the Junior Board of the Buffalo General Hospital, Buffalo, N. Y.

[1] Tillett, W. S., and Garner, R. L., *J. Exp. Med.*, 1933, **58**, 485; Garner, R. L., and Tillett, W. S., *J. Exp. Med.*, 1934, **60**, 239; Tillett, W. S., Edwards, L. B., and Garner, R. L., *J. Clin. Invest.*, 1934, **13**, 47; Tillett, W. S., *J. Clin. Invest.*, 1935, **14**, 276; Tillett, W. S., *J. Bact.*, 1935, **29**, 111.

[2] Van Deventer, J. K., and Reich, T., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 821.

by Dennis and Berberian,[3] Dart,[4] Witebsky and Neter.[5] Dennis and Berberian demonstrated such an anticoagulant also in a glucose-broth culture of one strain of *S. viridans.* These authors reported that type-specific (El) antiserum neutralized the El anticoagulant when used in amount of 0.2 cc. or more. They concluded that there is evidence that fibrinolysin and anticoagulant are antigenic and exhibit some degree of type-specificity. The following facts, however, seem to cast doubt on the conception of the antigenic or even type-specific nature of bacterial anticoagulants: (1) Normal serum and heterologous antisera inhibit the streptococcal anticoagulant to about the same degree as does streptococcal antiserum. (2) Normal spinal fluid, known to be rather poor in normal antibody-functions, counteracts anticoagulant as well as normal serum. We, therefore, believe that this type of neutralization of the anticoagulant is due to non-specific factors operative in serum and spinal fluid respectively. The lack of antigenicity of the streptococcal anticoagulant would harmonize with the recent findings of Dennis and Adham,[6] according to which the effective factor of the anticoagulant is primarily lactic acid, perhaps with a minor admixture of other organic acids.

The production of anticoagulant is by no means limited to streptococci; a wide variety of bacteria, *e. g.,* staphylococcus,[7] enterococcus, pneumococcus, *B. coli, B. Friedländer, B. pyocyaneus* and others may produce anticoagulants in glucose-broth.[5] The following report is concerned with a comparative study on the fibrinolytic and anticoagulating properties of both *S. hemolyticus* and enterococcus *(S. fecalis).* The microörganisms were obtained from human sources; the tests for the demonstration of fibrinolysin and anticoagulant were set up according to the original technic[1]: the supernatant fluid (in decreasing amounts, volume 0.5 cc.) of 18-hour plain infusion and 1% glucose-infusion-broth cultures respectively, were mixed with 1.0 cc. of 1:5 diluted, oxalated plasma. Then 0.25 cc. of 0.25% solution of calcium chloride in saline was added. The tubes were shaken thoroughly, incubated at 37°C., and read at various intervals: The fibrinolysin dissolves the plasma-clot; the anticoagulant prolongedly (24 hours) inhibits plasma-coagulation.

Sixty-seven strains of *S. hemolyticus* and 50 strains of enterococcus were examined. The results are summarized in Table I.

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[3] Dennis, E. W., and Berberian, D. A., *J. Exp. Med.,* 1934, **60**, 581.

[4] Dart, E. E., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 285.

[5] Witebsky, E., and Neter, E., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 858; Neter, E., and Witebsky, E., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 549.

[6] Dennis, E. W., and Adham, L. D., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 84.

[7] Neter, E., *J. Bact.,* 1937, **34**, 243.

Of 67 strains of *S. hemolyticus*, 61 (*ca.* 91%) produced fibrinolysin in plain infusion-broth, and 17 (*ca.* 25%) produced anticoagulant in 1% glucose-broth. In contrast, all 50 strains of enterococcus produced the anticoagulant in glucose-broth and failed to form the fibrinolysin. The high percentage of fibrinolytic strains of *S. hemolyticus* from human lesions is in accord with the findings of Tillett,[1] who reported that out of 157 strains, 98.1% were fibrinolytic. In a study of the relation of source to fibrinolytic activity of hemolytic streptococci, Madison[8] found 94% of strains isolated from internal lesions to be fibrinolytic, as contrasted with only 17% of strains isolated from superficial lesions. This latter figure was increased to 35% when the concentrative method was employed (Madison[9]).

In view of the different fibrinolytic and anticoagulating properties of *S. hemolyticus* and enterococcus, the question arises as to the respective properties of hemolytic enterococci. The lack of fibrinolytic activity of Lancefield's group D—representing hemolytic strains of the *S. fecalis* group—was reported by Lancefield and Hare,[10] Hare and Maxted.[11] The characteristics of different members of the *S. fecalis* group were extensively studied by Sherman and his coworkers.[12, 13, 14] Sherman, Stark and Mauer[13] also established the identity of Lancefield's group D with *S. zymogenes*.

Five strains of hemolytic enterococci were isolated in this laboratory from human sources and examined for fibrinolytic and anticoagulating properties. All failed to show fibrinolytic activity when tested according to the original technic, but produced anticoagulant in glucose-broth. Through the courtesy of Dr. Sherman, 4 more strains of hemolytic enterococci from human sources were obtained, 2 of them identified as *S. zymogenes,* the other 2 as *S. durans*. The lack of fibrinolytic activity was observed by Dr. Sherman (personal communication) when secondary growth was inhibited by incubating at 53°C. after coagulation had occurred. When tested in this laboratory, they were found to produce anticoagulant in glucose-broth, but failed to exhibit fibrinolytic activity. Thus, 9 strains of hemolytic enterococci produced the anticoagulant and failed to show fibrinolytic activity when tested with the original Tillett-Garner technic. Further experiments will deter-

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[8] Madison, R. R., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 1018.

[9] Madison, R. R., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 445.

[10] Lancefield, R. C., and Hare, R., *J. Exp. Med.*, 1935, **61**, 335.

[11] Hare, R., and Maxted, W. R., *J. Path. Bact.*, 1935, **41**, 513.

[12] Sherman, J. M., Mauer, J. C., and Stark, P., *J. Bact.*, 1937, **33**, 275.

[13] Sherman, J. M., Stark, P., and Mauer, J. C., *J. Bact.*, 1937, **33**, 483.

[14] Sherman, J. M., and Wing, H. N., *J. Dairy Science*, 1937, **20**, 165.

TABLE I.

Fibrinolytic and Anticoagulating Properties of *Streptococcus hemolyticus* and *Streptococcus fecalis* from Human Sources.

No. of Strains		Number of Strains Producing				Total:	
		Fibrinolysin* and Anticoagulant†	Fibrinolysin Only	Anticoagulant Only	Neither Fibrinolysin nor Anticoagulant	Fibrinolytic	Anticoagulant Strains
67	*Streptococcus hemolyticus*	13	48	4	2	61 (Ca 91%)	17 (Ca 25%)
50	*Enterococcus (Streptococcus fecalis)*	0	0	50	0	0	50 (100%)
9	*Enterococcus hemolyticus*	0	0	9	0	0	9

*Dissolution of plasma clot within 24 hr.

†Constant (24 hr.) inhibition of plasma coagulation.

mine whether the difference in fibrinolytic activity is qualitative or quantitative and whether a relation exists between the apparently constant production of anticoagulant in glucose-broth by hemolytic enterococci and the final low pH of the broth, characteristic of the *S. fecalis* group.

The anticoagulant of enterococci is not necessarily an artificial product occurring *in vitro* only. Recently it was shown[15] that the *Enterococcus hemolyticus* anticoagulant may occur *in vivo:* the fluid obtained from the lesion in a case of Ludwig's angina, caused by hemolytic enterococci showed anticoagulating properties as did the isolated strain when cultured in glucose-broth. Meanwhile, we found bacterial anticoagulants in 17 other exudates of various origin such as abscesses, empyemic fluid, etc. In 5 of them the culture revealed enterococci, which were found in pure culture in one case and in the remaining 4 cases together with *B. coli, B. welchii* and staphylococcus respectively. It cannot yet be decided whether the anticoagulating properties of such exudates are due to one factor or to different factors and whether the anticoagulant produced *in vivo* is identical with that *in vitro*. The demonstration of bacterial anticoagulants in natural infections supplements the recent finding of *S. hemolyticus* fibrinolysin in human lesions.[16]

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Chick Embryo Broth and Chick Embryos, Held in Cold Storage, as Sources of Growth Stimulants for Tissue Cultures.

Mary E. Shipp. (Introduced by F. H. Swett.)

From the Department of Anatomy, Duke University School of Medicine.

It is frequently difficult during the winter months in certain localities to obtain the adequate and inexpensive supply of fertile eggs so essential where constant supplies of embryo juice are required for procedures in tissue culture. Two methods were devised for utilizing chick embryos from which growth-promoting extracts might be made while a plentiful supply of embryos was available.

Embryo Broth. Five hundred grams of finely chopped embryos were steeped in one litre of Tyrode's solution for 24 hours in a refrigerator and then heated for 2 hours in an Arnold steam sterilizer. The resulting broth was strained through fine cheese cloth and as much fluid as possible forced from the cooked tissue. Ten grams of peptone and 5 gm. of sodium chloride were added, the whole filtered through paper and made up to one litre with Tyrode's solution and sterilized in an Arnold steamer for 30 minutes. This fluid was the concentrated stock solution. Portions of it were diluted with Tyrode's solution to make a 2% solution, adjusted to pH 7.6, sterilized in flasks in an Arnold steamer for 2 hours and stored at 30°F. in a refrigerator. The final fluid was used in lieu of fresh embryo juice.

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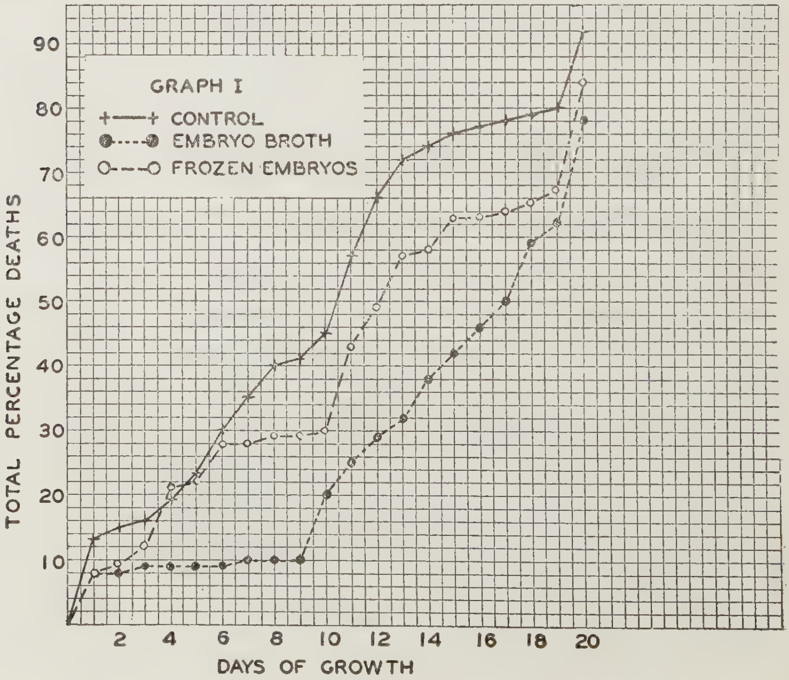
[15] Neter, E., and Young, G. S., *Am. J. Dis. Child.*, 1937, **53**, 1531; Neter, E., *Arch. Path.*, 1937, **23**, 295.

[16] Neter, E., and Witebsky, E., *J. Bact.*, 1936, **31**, 77; Neter, E., Proc. Soc. Exp. Biol. and Med., 1936, **34**, 735.

Frozen Embryos. Eight-day chicks were removed aseptically from their shell and placed in sterile section dishes—2 or more to a dish. These were placed in a larger, sterile, glass-covered container and stored at 10°F. in a refrigerator. From these frozen embryos, 25% embryo juice could be made in the usual manner at any time.

The broth and frozen embryos were kept in storage, as described, for 6 months and were thereafter tested for growth-promoting substances against embryo juice made from fresh 8-day embryos.

The cultures for the entire experiment were made by the cover-



slip-hanging-drop method. Aseptic precautions were observed during all manipulations and all water used was triply distilled in a pyrex glass apparatus. The plasma was obtained by centrifuging blood drawn, without the use of an anticoagulant, from the wing veins of young hens. The embryo juice (25%) for the control cultures was prepared by extracting eight-day chick embryos in Tyrode's solution (pH 7.5 to 7.6), containing dextrose.

For the first test series with the media, 129 cultures of 8-day chick heart were planted in equal parts of plasma and the prepared broth; thus, making a 1% concentration of broth in the planting medium. For the second test series, 145 cultures of heart tissue from 8-day chick embryos were planted, in the same manner, substituting for the broth, a 25% solution of embryo juice prepared from the frozen embryos. Control cultures, 180 in number, were prepared at the same time, using the same heart tissue, the same plasma and 25% embryo juice made from fresh 8-day embryos. All the cultures were incubated at 37°C. and a daily record of each was kept for 20 days. From the data collected on the control and from the 2 experimental series, average percentage death rate curves were constructed (Graph 1). With the use of a delineascope and planimeter the area of the original explant was measured

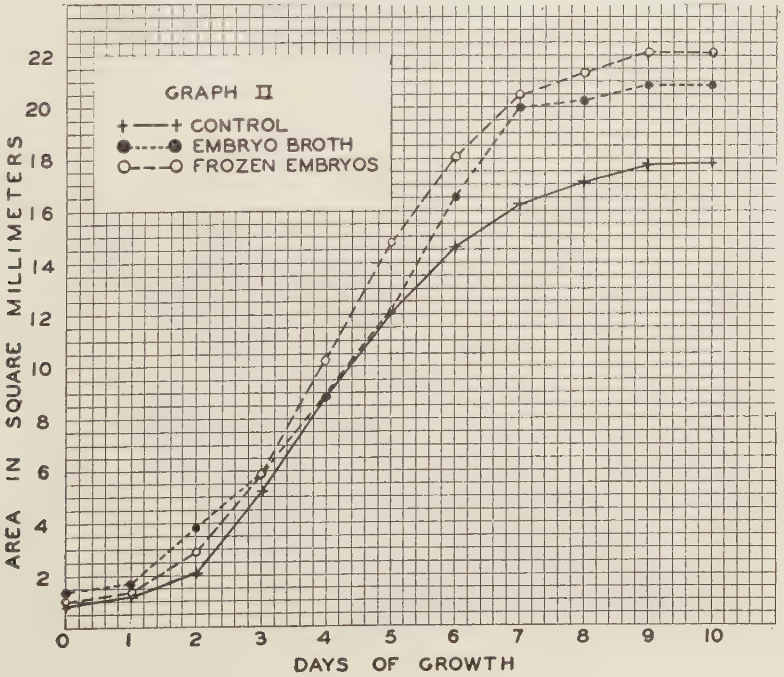


TABLE I.

No. of Days	Control			Frozen Embryos			1% Broth			Frozen Embryos vs. Control				1% Broth vs. Control			
	Means	Probable Error	Standard Deviation	Means	Probable Error	Standard Deviation	Means	Probable Error	Standard Deviation	σ_D*	D_M	$3\sigma_D$	Significant Difference	σ_D*	D_M	$3\sigma_D$	Significant Difference
O.T.	0.89	±.03	0.40	0.97	±.02	0.39	1.37	±.04	0.61	0.54	0.08	1.62	0	0.07	0.48	0.21	+
1	1.16	±.03	0.46	1.31	±.03	0.50	1.69	±.05	0.76	0.66	0.15	1.98	0	0.89	0.53	2.67	0
2	2.07	±.11	1.64	2.96	±.10	1.64	3.97	±.13	2.11	0.23	0.89	0.69	+	0.26	1.90	0.78	+
3	5.16	±.22	3.31	5.88	±.15	2.28	5.93	±.22	3.46	0.77	0.72	2.31	0	0.47	0.77	1.41	0
4	8.89	±.30	5.00	10.14	±.27	4.12	8.92	±.28	4.44	0.63	1.25	1.89	0	0.65	0.03	1.95	0
5	12.04	±.43	6.57	14.71	±.38	5.83	12.05	±.26	5.32	0.85	2.67	2.55	+	0.82	0.01	2.46	0
6	14.55	±.58	8.19	18.01	±.41	6.32	16.51	±.44	6.80	1.01	3.46	3.03	+	1.03	1.96	3.09	0
7	16.22	±.72	10.86	20.51	±.47	7.07	20.00	±.48	7.48	1.27	4.29	3.81	+	1.30	3.78	3.90	0
8	17.03	±.62	9.38	21.29	±.49	7.42	20.34	±.46	7.14	1.17	4.26	3.51	+	1.15	3.31	3.45	0
9	17.73	±.39	6.00	22.07	±.51	7.68	20.89	±.44	6.86	0.95	4.34	2.85	+	0.88	3.16	2.64	+
10	17.73	±.39	6.00	22.07	±.51	7.68	20.89	±.44	6.89	0.95	4.34	2.85	+	0.88	3.16	2.64	+

*σ_D, Standard Error of Difference of Means; D_M, Difference of Means; Significant Difference, $D > 3\sigma_D$.

and the daily outgrowth of each culture was recorded for a period of 10 days. At the close of the experiment, 109 cultures planted with broth; 105 planted with extract from frozen embryos; and 103 controls utilizing fresh embryo juice, had lived for 10 days or longer. These cultures form the basis for the results recorded in Table I and Graph 2.

Microscopical study of each of the cultures in the experimental series showed no marked differences or variations in the behavior of the cells or any structural changes which would differentiate these cells from those in the control series. Many dividing cells were observed and an abundant outgrowth took place in all the cultures. Graphs 1 and 2 are self-explanatory. Examination of Table I indicates that increased growth of tissue cultured in 1% chick embryo-broth, is not sufficiently greater than that of tissue grown in fresh embryo juice to make the former medium of any particular value except as a matter of convenience during periods when fresh embryos are scarce. On the other hand the difference in growth of cultures utilizing embryo juice made from frozen embryos compared with the growth of cultures in juice from fresh embryos, is so much greater as to be of real significance. Furthermore, the use of juice made from frozen embryos precludes the possible contamination of cultures due to the introduction of viable cells from insufficiently centrifuged fresh extracts.

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The Biuret and Ninhydrin Tests for Proteins as Measured with Hardy's Spectrophotometer.

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Color tests for proteins have long been in use, although in most cases the chemistry involved is not clearly understood. One method of attack upon this problem is to analyse the transmission spectra of the colored solutions with the aid of Hardy's recording photoelectric spectrophotometer. This has been done for the ninhydrin and biuret reactions.

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*Contribution No. 107 from the Department of Biology and Public Health, Massachusetts Institute of Technology, Cambridge, Mass.

The ninhydrin test was performed according to Koch[1] by adding 2.5 cc. 0.1% ninhydrin to 10 cc. protein solution, and boiling for one minute. The cloudy blue to red solutions were filtered before measuring their transmission of light in the various regions of the spectrum. For the ninhydrin test on 10% bacto-peptone (Fig. 1, upper curve) the percent transmission of light by the colored solution increases continuously from 2% at a wavelength of 400 mμ to 88% at 700 mμ. A very similar curve is obtained for the ninhydrin test

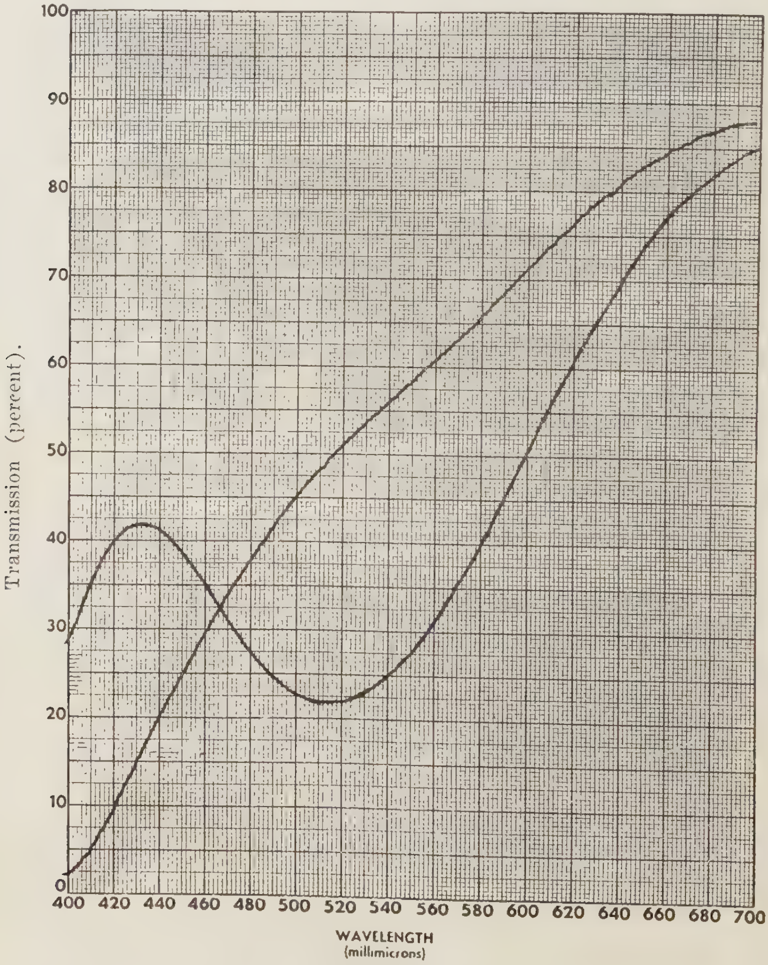

FIG. 1.

Upper curve: Transmission spectrum for the ninhydrin test on 10% bacto-peptone.

Lower curve: Transmission spectrum for the biuret test on 20% casein.

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[1] Koch, F. C., *Practical Methods in Biochemistry*, Baltimore, 1937.

on 20% casein (Hammarsten). Digestion of these 2 proteins by 0.25% trypsin (Fairchild and Foster) at about pH 8 and at 50°C. caused very little change in the shape of the curve, with a slight decrease in percent transmission over the middle of the spectrum. In all cases where digestion was studied, the course of the reaction was followed by means of the formal titration. Curves for the increase in carboxyl groups as a function of time were very similar to those obtained by Northrop and Kunitz.[2]

The biuret reaction was performed according to Koch[1] by adding 5 cc. 10% NaOH to 5 cc. protein solution, and then adding 20 drops 1% $CuSO_4$. The cloudy or clear red to purple solutions were filtered before analyzing with the spectrophotometer. The test was performed on 2 preparations of 20% casein, a 10% solution of bactopeptone, 3.5 and 5% egg albumen, and 5% edestin. Similarly shaped colorimeter curves were obtained for all these proteins with a maximum at 431 mμ, a minimum at 514 mμ, and a second maximum at about 700 mμ (Fig. 1, lower curve). The actual percent transmission at a given wave length, and also the shape of the curve, varies with the nature and concentration of the respective proteins.

The biuret reaction was also performed according to Lieben and Jesserer[3] by adding 6 cc. 10% NaOH plus 4 cc. 10% $CuSO_4$ to 10 cc. 1% protein. The heavy precipitate was removed by filtering. Only the 2 egg albumen and the edestin solutions were used. The shape of the colorimeter curves was very similar to that obtained for the Koch biuret. The transmission curves are somewhat displaced toward the region of higher wave lengths so that the first maximum now comes at 437 mμ, the minimum at 552 mμ, while the second maximum is off the scale. The intensity and shade of color in the biuret reaction are similar but not identical for different proteins at equal concentrations, although Lieben and Jesserer[3] believe they are the same.

The spectrophotometer reveals the fact that the violet biuret color is made up of 2 components, red and blue. By decreasing the amount of 1% $CuSO_4$ added to 2.5% egg albumin in 5% NaOH from 40 to 10 drops the blue component of the percent transmission curve practically disappears. The red component, however, is almost unaffected by this procedure. It is suggested that the red color is due to the presence of a copper-protein complex, while the blue color may be caused by the copper ions left free in the solution. The similarity between the blue portion of the curve and the percent transmission curve obtained with a saturated $CuSO_4$ solution supports this suggestion. From a study of extinction coefficients Lieben and Jesserer[3] also concluded that the biuret color consisted of red and blue components. They also report that if the biuret mixture is carefully acidified, the red component disappears before the blue.

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[2] Northrop, J. H., and Kunitz, M., *J. Gen. Physiol.*, 1932, **16**, 295.

[3] Lieben, F., and Jesserer, H., *Biochem. Z.*, 1936, **285**, 36.

The biuret test is specific for peptide linkages in proteins, and is also given by such compounds as biuret and malonamide (Schiff[4]). A typical protein biuret curve was obtained with maxima at 420 and 700 mμ, and a minimum at 520 mμ, for a solution containing 1.25% biuret, 5% NaOH, and 30 drops 1% $CuSO_4$. A similar solution containing 2.5% malonamide gave a blue maximum at 445 mμ, but the red portion of the curve was almost completely missing. Biuret apparently represents the type of grouping in proteins responsible for the biuret test better than does malonamide.

The digestion of 20% casein, 10% bacto-peptone, 3.5 and 5% egg albumen, and 5% edestin by 0.25% trypsin was studied at 50°C. and pH about 8. The formal titration curves were similar to those obtained in the ninhydrin experiments. Biuret tests were performed on the digest at successive intervals and analyzed with the spectrophotometer. A comparison of the transmission curves of the biuret tests indicated that during the course of digestion there is relatively no change in the positions of the 2 maxima and the minimum. There was considerable fluctuation in the percent transmission of the biuret solution at a given wave length, however. These fluctuations during digestion were more marked in the blue portion of the curve than in the red. There was no consistent increase or decrease in percent transmission during hydrolysis, when the biuret test was performed by the 2 different methods. The cause of the sharp rise or fall in percent transmission in various parts of the spectrum during digestion is not known. Changes in the colloidal properties of the protein solution might possibly be responsible.

Different proteins give essentially the same ninhydrin and biuret tests as measured with the spectrophotometer, and the general shape of the transmission curves is not changed by tryptic digestion of the proteins.

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[4] Schiff, H., *Berichte,* 1896, **29**, 298, 354.

9471 P

Observations on the Locus of Deamination.*

Morton F. Mason. (Introduced by C. S. Robinson.)

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It has been shown by Kisch[1, 2] that both the oxygen consumption and ammonia production of surviving slices of rat kidney cortex are greater than that of liver slices in the presence of various added amino acids. Krebs[3, 4] has also shown that kidney cortex is the most actively deaminating tissue per unit weight by use of the tissue slice technique. These data suggest that in spite of the much larger mass of liver tissue, the kidneys may participate to a considerable extent in deamination occurring in the normal intact animal. It is the purpose of this report to give the results of experiments designed to measure deaminative activity by the kidney of the intact, unanesthetized dog.

The dogs employed were prepared several months previously by unilateral nephrectomy with explantation of the remaining kidney into the flank by the technique of Rhoads.[5] At the time of the experiment blood samples were drawn simultaneously from the renal vein and femoral artery without aid of anesthesia. Samples were taken under fasting conditions and at various times following feeding of from one to 2 pounds of lean meat. These samples were analyzed for amino acid amino nitrogen. This determination was performed on picrate filtrates of whole blood or serum by the manometric measurement of CO_2 evolved from the carboxyl groups of the amino acids upon heating with an excess of ninhydrin.[6] The details of this method which seems more specific than those methods based on the determination of primary amino nitrogen will be given in a subsequent publication. The values of amino acid amino nitrogen were calculated on the assumption that one millimole of CO_2 is equivalent to 14 mg. amino acid amino nitrogen. The fact that this

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* Aided by a grant to the Vanderbilt University School of Medicine from the Division of Medical Sciences of the Rockefeller Foundation.

[1] Kisch, Bruno., *Biochem. Z.*, 1935, **280**, 41.

[2] Kisch, Bruno., *Biochem. Z.*, 1935, **280**, 55.

[3] Krebs, H. A., *Biochem. J.*, 1935, **29**, 1620.

[4] Krebs, H. A., *Chem. and Ind.*, 1935, **54**, 172.

[5] Rhoads, C. P., *Am. J. Physiol.*, 1934, **109**, 324.

[6] Van Slyke, D. D., and Dillon, R. T., Proc. Soc. Exp. Biol. and Med., 1936, **34**, 362.

TABLE I.

Dog	Whole Blood or Serum	Fasting Amino Acid —NH_2N Arterial mg.%	Fasting Amino Acid —NH_2N Renal Vein mg.%	Post-prandial Amino Acid —NH_2N Arterial	Post-prandial Amino Acid —NH_2N Renal Vein	Time of drawing of post-prandial blood samples
1	whole blood	5.43	5.50	—	—	—
1	serum	—	—	5.58	5.59	3 hr. after 1 lb. of meat.
2	,,	3.63	3.53	7.41	7.98	6 ,, ,, 2 ,, ,, ,,
2	,,	—	—	6.84	6.68	5 ,, ,, 1 ,, ,, ,,
3	,,	2.65	2.63	5.34	5.41	5 ,, ,, 2 ,, ,, ,,
4	whole blood	5.07	5.00	7.37	7.46	6 ,, ,, ,, ,, ,, ,,
5	,, ,,	4.45	4.56	8.18	8.33	4½ ,, ,, ,, ,, ,, ,,
6	serum	3.63	3.74	—	—	—
7	,,	3.85	4.09	5.89	5.84	3 ,, ,, ,, ,, ,, ,,
7	whole blood	—	—	6.73	6.84	9½ ,, ,, ,, ,, ,, ,,

assumption is not strictly true is immaterial as far as the conclusions to be drawn from these results are concerned.

The results of 10 experiments performed on 7 dogs are given in Table I. Neither in the fasting state nor at various intervals following feeding of meat was there any appreciable consistent arterio-renal venous difference in amino acid amino nitrogen in either serum or whole blood. It can be calculated that if as much as 10% of the total deamination of absorbed amino acids took place in the kidney, an arterio-renal venous difference in amino acid amino nitrogen would be readily detected by the method employed. It seems likely that sufficiently long intervals had elapsed after feeding to make certain that an active metabolism of the absorbed amino acids was taking place at the time blood samples were taken, *e. g.*, specific dynamic action is exhibited within the limits of such periods. Further experiments including longer post-absorptive periods prior to sampling, similar experiments following intravenous injection of individual amino acids, and the effects of systemic acidosis will be reported later.

These results indicate that the kidney plays a minor rôle as a locus of deamination in intact unanesthetized dogs under the conditions of these experiments. Presumably this physiological function is performed almost entirely in the liver[7] in accordance with classic theory, although the results of London[8] indicate that considerable deamination may take place in the intestinal wall. Whether or not the mechanisms for deamination existing in the kidney take over this function to a quantitatively large degree under certain unusual circumstances remains to be investigated.

Conclusions. The absence of a demonstrable arterio-renal venous difference in amino acid amino nitrogen at various intervals following feeding of meat to dogs indicates that the kidney does not play a *quantitatively* important rôle in the deamination of absorbed amino acids in the normal intact animal.

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[7] Neber, M., *Z. Physiol. Chem.*, 1936, **240**, 59.

[8] London, E. S., Dubinsky, A. M., Wassilewskaja, N. L., and Prochorowa, M. J., *Z. Physiol. Chem.*, 1934, **227**, 223.

9472

Oxidation-Reduction Potentials of Certain Synthetic Flavins.

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The electrochemical properties of natural and synthetic lactoflavin and their relation to chemical structure as well as to biological activity have been well established. Furthermore, the oxidation-reduction potentials, E'_0, of a large series of substituted flavins have been determined by Kuhn and Boulanger.[1] They found that the index potentials, E_i, are greater than 14 millivolts which would correspond to 2 electron transfer, and less than 28 millivolts which would correspond to one electron transfer. This suggested a semiquinone-formation. Michaelis and coworkers[2] demonstrated the presence of a colored semiquinone over the entire pH range studied, and determined its formation-constant, quantitatively. While the work of these investigators was in progress, the present authors were independently studying the oxidation-reduction potentials of synthetic lactoflavin and 2 substituted flavins prepared essentially according to the methods described by Karrer.[3] The results of our experiments are briefly summarized in Table I, in which E'_0 is the normal potential at the stated pH, and E_i designates the index potential.

Although data on our substituted flavins, (7-mono-methyl-d-arabinose-flavin and 7-mono-methyl-l-arabinose-flavin), have not been previously reported, they are to be regarded merely as confirming Kuhn and Boulanger's findings that the potentials are largely independent of the sugar attached to the alloxazin ring and that they are only slightly shifted to the positive side if one of the 2 methyl groups is removed. Our data on the potential of lactoflavin are in essential agreement with those previously given for natural and synthetic lactoflavin.[1, 2, 4, 5] In agreement with the results of Michaelis, Schubert and Smythe,[2] we also found that the titration curve of the lactoflavin is less steep than would be expected on the basis of a

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* Supported in part by a grant from the Milton Fund.

[1] Kuhn, R., and Boulanger, P., *Ber. chem. Ges.*, 1936, **69**, 1557.

[2] Michaelis, L., Schubert, M. P., Smythe, C. V., *J. Biol. Chem.*, 1936, **116**, 587.

[3] Karrer, P., Beeker, B., Benz, F., Frei, P., Salomon, H., Schöpp, K., *Helv. Chim. Act.*, 1935, **18**, 1435.

[4] Barron, E. S. G., and Hastings, A. B., *J. Biol. Chem., (Proc.)*, 1934, **105**, vii.

[5] Stare, F. S., *J. Biol. Chem.*, 1935, **112**, 223.

TABLE I.

6,7-Dimethyl-d-ribose-flavin (Lactoflavin).

pH	Buffer	E'_0 (mv)	E_i (mv)	Oxidant (O) or Reductant (R) Used	
1.13	n/10 HCl	+122	19	(R) $TiCl_3$	
4.52	Acetate	— 86	18	(R) "	
4.52	"	— 88	17	(O) Benzo quinone	++
6.94	Phosphate	—207	16	(R) $Na_2S_2O_4$	
6.94	"	—207	16	(O) Naphthol-sulfonate-indophenol	+
6.98	"	—205	18	(R) $Na_2S_2O_4$	
6.98	"	—205	18	(O) Dichlorophenol-indophenol	+
7.68	"	—223	17	(R) $Na_2S_2O_4$	
7.68	"	—223	18	(O) Dichlorophenol-indophenol	+
10.52	"	—343	18	(R) $Na_2S_2O_4$	
10.52	"	—343	18	(O) Naphthol-sulfonate-indophenol	+
10.99	"	—365	18	(R) $Na_2S_2O_4$	
10.99	"	—365	18	(O) Naphthol-sulfonate-indophenol	+
11.41	"	—399	17	(R) $Na_2S_2O_4$	

7-Mono-methyl-d-arabinose-flavin

pH	Buffer	E'_0 (mv)	E_i (mv)	Oxidant (O) or Reductant (R) Used	
1.13	n/10 HCl	+132	17	(R) $TiCl_3$	
4.52	Acetate	— 74	16	(R) "	
5.61	Phosphate	—141	14	(O) Dichlorophenol-indophenol	++
6.18	"	—164	15	(O) " "	++
6.91	"	—192	16	(O) " "	++
7.70	"	—216	16	(O) " "	++
11.41	"	—376	16	(O) Naphthol-sulfonate-indophenol	++

7-Mono-methyl-l-arabinose-flavin

pH	Buffer	E'_0 (mv)	E_i (mv)	Oxidant (O) or Reductant (R) Used	
4.52	Acetate	— 77	16	(R) $TiCl_3$	
6.41	Phosphate	—174	16	(O) Naphthol-sulfonate-indophenol	++
7.71	"	—213	16	(O) " " "	++
10.59	"	—324	17	(O) " " "	++

+ Solution reduced by preceding titration.
++ Solution previously reduced by H_2 and colloidal palladium.

2-electron transfer, over the entire range of pH studied, suggesting a semiquinone formation. The potentials of 7-methyl-d-arabinose-flavin are only slightly more positive than those of lactoflavin, the index potential being less than that of lactoflavin. The potentials of 7-methyl-d-arabinose-flavin are essentially identical with those of the l-arabinose compound. The dissociation constants of the substances titrated are practically the same as indicated by the curves of E'_0 against pH.

Due to the fact that the amounts of material available were quite limited, a vessel was devised which permitted the titration of 0.6 mg. of flavin dissolved in 10 cc. of buffer. In some cases, the curve of oxidation was determined on the same solution as the curve of reduction. Good agreement was found between the two curves. The oxidants and reductants used are indicated in the table.

9473 P

Situs Cordis in Parabiotic Frog Larvæ.

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It is known that the situs of the heart and viscera can be experimentally reversed by turning around a portion of the archenteric roof in the open neural plate stage of development.[1] Spemann and Falkenberg[2] have also shown that if salamander eggs are divided into 2 by a hair loop, the sinistral larvae which result all have normal situs while some of the dextral ones have *situs inversus*. Spontaneous double monsters in man as well as in some of the lower vertebrates occasionally show *situs inversus* of the heart and viscera of the right member of the pair (A component).[3] Politzer,[4] however, reports 2 cases of spontaneous twinning in Salamandra in which *situs inversus* occurred in the left member (B component). Swett[5] has also reported *situs inversus viscerum* in the left member of double trout. Moreover, in some spontaneous dicephalus monsters of *Rana sylvatica* discovered some years ago, the hearts of the left individuals were in inverse situs, while the right hearts were of normal asymmetry.

This paper is a preliminary report of an attempt to study the question further. Parabiotic twins were made from *Rana pipiens* embryos in open neural plate stage by removing the ectoderm, and in some cases the mesoderm as well, from a portion of the lateral body wall and holding the embryos together until healing took place. Fifty-eight pairs were available for study at the feeding stage.

Although in most cases nothing but the ectoderm had been removed in making the twins, and the 2 hearts lay in a common pericardium with the *sinus venosus* of each animal communicating with that of the other, the right ventricle was larger than the left in 36 cases. The ventricles were the same size in 6, and the left ventricle larger in only 11 cases.

Four pairs had a single heart, evidently formed by the fusion of the 2 anlagen. The right heart was of normal asymmetry in all the other cases. The asymmetry of the left heart could be determined with certainty in only 43 cases. In 20 of these, in which the medial sides of the 2 heads were fused and the medial gills consequently completely absent, the left heart was in normal situs. The heads were farther apart in 23 cases so that gills were present on the medial sides. In 14 pairs the left heart was also in normal situs, but in 9 pairs *situs inversus cordis* was present in the left individual (Fig. 1). The anterior extent of fusion on the medial side has, therefore, an important bearing on the asymmetry of the heart.

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[1] Pressler, K., *Arch. f. Entw.-Mech.*, 1911, **32**, 1.

[2] Spemann, H., and Falkenberg, *Arch. f. Entw.-Mech.*, 1919, **45**, 371.

[3] Morrill, C. V., *Anat. Rec.*, 1919, **16**, 265.

[4] Politzer, G., *Arch. f. Entw.-Mech.*, 1926, **108**, 417.

[5] Swett, F. H., *Anat. Rec.*, 1921, **22**, 183.

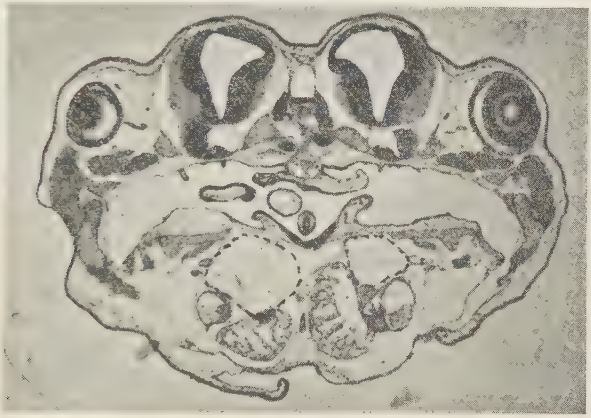

FIG. 1.

Cross section of parabiotic twin showing mirror imaging of the 2 hearts. The thin walled atria have been indicated by dotted lines. Below them lie the ventricles with trabeculæ carneæ. The conus arteriosus of each heart is the round chamber on the lateral side of the atrium and ventricle. Note that the right heart is in normal situs, while the left is in situs inversus.

The 2 livers were usually fused together and usually lay in the right side of the common body cavity. Two gall bladders were almost always present in the fused liver. In one case in which the livers were not fused, *situs inversus viscerum* as well as *situs inversus cordis* was present in the left individual. The double stomach and intestine of all the other cases were in normal situs with respect to the common liver.

Summary, The right heart of parabiotic twins of frog larvae is usually larger than the left. If medial gills are absent, both hearts are of normal asymmetry. If medial gills are present, the left heart is of inverse situs in some cases. The situs of the viscera is as a rule not affected by parabiosis.

9474

Explanation *In Vitro* of Transverse Pieces of Early Rat Embryos.

J. S. Nicholas and Dorothea Rudnick.

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The primitive axis of the rat embryo appears during the ninth day after insemination, on the inner concavity of the upper or antimesometrial portion of the blastodermic vesicle. Accordingly its sagittal section is U-shaped. In order to isolate definite portions of the axis, the embryonic end of the cylinder is first cut off from the rest by a transection in the region of the amnion; then the embryo is flattened by means of a slit in each lateral wall of the vesicle so isolated. Transverse cuts may now be made at any level of the axis. In the present series the whole posterior half was isolated as a single explant, the anterior half usually being divided into thirds transversely.

The isolated fragments of the embryonic regions of the rat in stages just previous to and during the formation of the embryonic axis are capable of typical differentiation *in vitro.* The pieces were explanted on coverslip clots of rat plasma and rat embryo or placental extract. The plasma donor was injected with a few cc. of 10% dextrose immediately before the bleeding. The cultures were usually washed or transferred after 48 hours; fixed after 3 or 4 days, photographed *in toto* and later examined in serial sections.

The designated levels of embryos in stage 14 (medullary plate) and stage 15 (head-fold) round up into little vesicular structures, and differentiate characteristic primitive organs. The anteriormost third of the anterior part of the axis always includes part of the yolk-sac which forms its typical epithelium, blood islands and vascular channels. One or two pulsating myocardial masses invariably develop; histologically these may show little differentiation. Mesenchyme frequently is found; less frequently medullary tube. The second third of the anterior axis usually forms yolk-sac, and always pulsating heart-masses. In one case, a medullary tube and entoderm invaginating as though forming foregut were recognized. The third piece, derived from the part of the anterior axis nearest the antimesometrial pole of the capsule, never forms yolk-sac. Medullary tube, mesenchyme, and in one case a heart-mass, are the structures found in such explants.

The posterior half of the embryo yields a complex vesicular mass composed of yolk-sac, outgrowths of allantoic origin, and much embryonic material. Medullary tubes, occasionally with chorda, somites, dense mesenchyme and differentiating muscle, lateral plate material split to form coelomic cavities, and possibly nephric tubules, are found in such masses.

Evidently at this stage, the levels of the axis are all equally capable of differentiation *in vitro* to form primitive axial structures of characteristic level. Histogenesis of both medullary and muscular elements may proceed quite normally.

A few cases of explants of pieces in the primitive streak stage (stage 13) are available. The anteriormost third of the anterior half may form pulsating heart tissue and yolk-sac epithelium. The entire anterior half may form well-differentiated lobed brain tube, foregut, heart and mesenchyme. Only one explant of the posterior half at this stage was successful; it contained yolk-sac and a necrotic outgrowth probably allantoic in nature. The posterior half of the primitive streak stage appears deficient in potency when compared with the anterior half of the embryo which may develop quite as well as the same material in later stages.

9475

Toxicity of Sulfanilamide. A Study of the Pathological Lesions in White Mice.*

P. O. Hageman. (Introduced by Francis G. Blake.)

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Since the introduction of sulfanilamide and related substances as chemotherapeutic agents, there have appeared in the literature several reports on their toxicity for experimental animals. Domagk[1] observed that 0.5 gm. per kilo of 'Prontosil Insoluble,'† given orally was well tolerated by mice and dogs, whereas the cat tolerated only 0.2 gm. per kilo. The urine of dogs which were exposed to varying amounts of 'Prontosil Insoluble' over a period of 14 days showed no red or white blood cells or casts. Similar studies were carried on by Buttle, Gray and Stephenson,[2] who found oral dosages of 0.6 gm. per kilo of 'Prontosil Insoluble' innocuous for mice. Larger doses, 1.25 and 5 gm. per kilo proved fatal in the majority of instances. These investigators also studied the toxicity for mice of sulfanilamide given orally and found 2.5 gm. per kilo well tolerated, whereas 5 gm. per kilo killed 2 of 6 animals and 10 gm. per kilo killed 6 out of 6. Rosenthal,[3] studying the toxicity of sulfanilamide for mice, reported 6 gm. per kilo as the fatal dose when given subcutaneously in olive oil. Two gm. per kilo produced spastic extremities, flexion of the spine, excitability and incoördination which disappeared after 12 hours. In a similar manner, Raiziss and his coworkers[4] found 1-2 gm. of sulfanilamide given subcutaneously to be well tolerated by mice, whereas 2.5 and 3 gm. proved to be a fatal dose. Maximum tolerated oral dosage for rabbits was found to be 1.5 gm. per kilo.

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* This study was made possible by a grant from the Clinical Research and Teaching Fund, Yale University School of Medicine.

[1] Domagk, G., *Deutsche. Med. Wchnschr.*, 1935, **61**, 250.

† Hydrochloride of 4-sulfamido-2,4-diamino azo benzene.

Up to the present time all published data relevant to toxicity of sulfanilamide for animals has presented maximum tolerated and lethal dosages. No studies of gross or microscopic pathology produced by sulfanilamide in animals have appeared in the literature.

The present report deals with the histology of various organs of mice exposed to varying doses of sulfanilamide.

Normal Swiss mice of approximately 20 gm. each in weight in groups of 5, were given large parenteral doses of Prontosil or sulfanilamide over a period of 14 days (Table I). At the end of this time, one of the surviving animals of each group was sacrificed and the remaining animals at weekly intervals thereafter. Five normal mice were studied as controls. Microscopic sections‡ of liver, spleen, kidney and vertebral and femoral bone marrow were made at the time each animal expired or was sacrificed. Mice to be sacrificed were given a lethal dose of ether in each instance. Tissues were fixed in Zenker-Formol and stained with hematoxylin and eosin unless otherwise indicated.

Results. 'Prontosil' was well tolerated in the dose given. The rapid absorption of the dye was evidenced by the fact that the ears became pink 5-10 minutes after the injection. Stools were dark red in color for the first 6-8 hours after injection, whereas the urine remained faintly pink as long as 18 hours. No reactions to the drug were noted.

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[2] Buttle, G., Gray, W., and Stephenson, D., *Lancet*, 1936, **1**, 1286.

[3] Rosenthal, S., *Pub. Health Rep.*, 1937, **52**, 48.

[4] Raiziss, G. W., Severac, M., and Moetsch, J., *J. Chemotherapy*, 1937, **14**, 1.

‡ The author is indebted to H. A. Weiner, M.D., for assistance in interpretation of the pathological material.

TABLE I.

Exposure of Normal 20 Gram Swiss Mice to "Prontosil" and Sulfanilamide.

Mouse No.	Dosage—Sulfanilamide					Time Sections Were Made		Hemosiderin in Spleen	Eosinophils in Bone Marrow
	mg. per day	Route	Days	gm./kg./day	Total mg.	Death—Day of Exposure	Sacrificed—Weeks after onset of Exposure		
1	....	....	....	....	....	....	....	—	+
2	....	....	....	....	....	....	....	—	+
3	....	....	....	....	....	....	....	—	+
4	....	....	....	....	....	....	....	—	+
5	....	....	....	....	....	....	....	—	+
6	50*	i.p.†	14	2.5	700	....	2	—	+++
7	50*	”	14	2.5	700	....	3	....	++
8	50*	”	14	2.5	700	....	D-4‡	++	++
9	50*	”	14	2.5	700	....	5	±	++++
10	50*	”	14	2.5	700	....	6	+	++
11	20	”	14	1	280	....	2	±	+++
12	20	”	14	1	280	....	3	±	++++
13	20	”	14	1	280	....	4	+	+++
14	20	”	14	1	280	....	5	++	+++
15	20	”	14	1	280	....	6	++	+++
16	30	subq.§	3	1.5	90	4	....	++	+++
17	30	”	5	1.5	150	6	....	++	+++
18	30	”	14	1.5	420	....	3	++++	++++
19	30	”	14	1.5	420	....	4	+++	+++
20	30	”	14	1.5	420	....	5	++++	+++
21	50	”	1	2.5	50	1	....	....	....
22	50	”	4	2.5	200	5	....	±	+++
23	50	”	7	2.5	350	8	....	±	+++
24	50	”	7	2.5	350	8	....	±	+++
25	50	”	10	2.5	500	11	....	++++	++++

*"Prontosil"—Disodium salt of 4-sulfamido-phenyl-2-azo-7 acetylamino-1-hydroxy-naphthalene-3,6 disulfonic acid.

†i.p.—intraperitoneal.

‡Died of mouse typhoid infection,

§subq.—subcutaneous.

Sulfanilamide was not well tolerated in large doses and produced unsteadiness, incoördination, paralysis, acute anterior flexion of the spine, spastic extension of the legs, prostration, convulsions, and sometimes death. With the smaller dosages the above symptoms were transient, coming on 30-60 minutes after the injection and disappearing in 12-18 hours.

Mice receiving sulfanilamide intraperitoneally suspended in saline showed a fibroblastic foreign body reaction with crystals scattered through the area of reaction. These areas appear as milky white spots on the surfaces of the liver, spleen and peritoneum.

Liver: sections of this organ showed no definite abnormalities.

Kidneys: no pathological changes were found.

Spleen: golden-yellow pigment in varying amounts was noted in areas adjacent to the malpighian bodies. By the aid of Turnbull's stain, this pigment was proven to be hemosiderin. As will be seen in Table I, the amount of hemosiderin was roughly proportional to the size of the total dose and the duration of life after onset of exposure. This suggests that the reaction was progressive after the drug was discontinued.

Bone marrow: A considerably greater incidence of eosinophils was found in the marrows of the animals exposed to 'Prontosil' or sulfanilamide than in the controls. No other differences were noted. Femoral and vertebral marrows were essentially identical in every instance.

Under the above experimental conditions it appears that sulfanilamide and 'Prontosil' exerted no definite toxic action on the liver or kidney.

The occurrence of hemosiderin in the spleen of animals exposed to various amounts of sulfanilamide suggests that increased blood destruction had taken place. Blood counts were not done in this series. Such a mechanism may explain the anemia seen clinically during prolonged sulfanilamide administration.

The greater number of eosinophils noted in the bone marrows of mice exposed to sulfanilamide and 'Prontosil' may possibly indicate an allergic response. If this is true it might be interpreted as further evidence in favor of the antigenic nature of sulfanilamide as suggested in a report on drug fever[5] following sulfanilamide.

These observations are preliminary but should serve as a guide for more complete and thorough studies in the future.

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[5] Hageman, P. O., and Blake, Francis G., *J. A. M. A.*, 1937, **109**, 642.

9476 P

Modification of the Site of Paralysis in Experimental Poliomyelitis.*

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From the Department of Anatomy, Johns Hopkins Medical School.

Römer[1] noted that monkeys inoculated intracranially with poliomyelitis virus frequently showed paralysis on the opposite side of the body. This suggested the participation of some decussating pathway in the propagation of the virus, but threw no light on the actual mechanisms which might be thus involved. A demonstration that virus may be routed along known fiber tracts in the central nervous system would go far toward explaining the fact that although the infective agent may enter through the olfactory tract, it usually traverses the entire brain stem without causing serious damage above the cord. Various cytologically distinct areas of the cerebral cortex provide the ideal conditions for such an experiment since their fiber connections with lower centers are sufficiently well known to provide a ready interpretation of the results obtained.

Accordingly 3 representative cortical areas were selected as sites for the introduction of the virus. The first, the so-called motor cortex, known cytologically as area 4 of Brodmann, was chosen because its known lower connections both ascending and descending are predominantly crossed. Next, the visual cortex, area 17 of Brodmann, presented the picture of an area with uncrossed connections. Its relations are with the lateral geniculate body of the same side, a region receiving the optic tracts, but possessing no direct connections with the lower brain stem or cord. The third region selected was the pre-motor cortex, or area 6 of Brodmann. The course of the fibers connecting this field with lower centers is not so well known as in the case of the other cortical areas. It is certain that its descending fibers run outside the pyramidal tract, proceeding as far as the midbrain and pons.[2] Stimulation of area 6 with the cortico-spinal tract eliminated[3] indicates that it is primarily concerned with movements of both arms, although there is a preponderance of crossed representation. Leg movements are

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* Supported by a grant from the President's Birthday Ball Committee for Infantile Paralysis Research.

[1] Römer, P. H., *Epidemic Infantile Paralysis*, New York, 1913.

[2] Levin, P. M., *J. Comp. Neurol.*, 1936, **63**, 369.

[3] Tower, S. S. Personal communication.

evoked to a lesser degree, but are obtained on both sides from the cortex of the one side. Area 6 then seems to present a diffusely stimulable surface in which there is no sharp localization for isolated muscles on the opposite side of the body as in the case of area 4.

Thirty-six animals were inoculated; 8 in the visual cortex (area 17), 16 in area 4, and 12 in area 6. The dosage in most cases was 0.2 cc. of 25% suspension of monkey cord containing MV virus. In each case the cortex was exposed and in areas 4 and 6 the regions were delimited by 60 cycle stimulation. The virus suspension was introduced through a 20-gauge needle with a guard to prevent accidental penetration into subcortical tissue. All the animals developed typical poliomyelitis, the paralysis appearing in 5-14 days. The average time elapsed from the date of inoculation until the appearance of paralysis was 7.6 days for area 4, 8.4 days for area 6, and 8.8 days for the visual cortex. The shorter incubation time for the area 4 inoculations may reflect the greater directness of its connections with the spinal cord.

Striking differences in the localization of initial paralysis were noted after inoculation into the three different areas. A crossed initial paralysis appeared in 87% of the inoculations into area 4 (14 cases) with a 13% incidence of uncrossed paralysis (2 cases). The visual cortex inoculations yielded 50% uncrossed paralyses (4 cases) while the remainder were either crossed or balanced on both sides. Inoculation of area 6 gave a little more than half the paralyses on the opposite side (58%) and the remainder either uncrossed or balanced. The experimental data thus correlate amazingly with anatomical and physiological knowledge of the connections involved.

It is possible to make interesting, though at the present time limited, inferences from the incidence of arm and leg paralyses. Inoculation of the visual cortex always gave isolated arm paralysis, with but one instance out of 8 in which the legs also were involved. Likewise area 6 inoculations gave initial arm paralysis in every case but one of the 12, but involved both arms and legs in 5 cases (41%). The sixteen area 4 inoculations were made into a region giving movements of leg or foot upon electric stimulation, and here the incidence of arm paralysis was lower (71%) while the leg paralyses were much more numerous than in other groups (64%). In 4 cases the opposite leg was paralyzed without the arm. Such findings point to a more direct type of virus propagation than that seen after the inoculation of areas 17 and 6. The involvement of the legs without arms suggests that the virus passed through the cervical cord on some system destined to end in the lumbar region. Naturally one thinks of the pyramidal tract in this connection, but it does not represent the only decussating connection with area 4. Thalamocortical fibers also end in the region inoculated, so that a relay from the thalamus to the cord via the spino-thalamic tracts or medial lemniscus must also be regarded as a possibility. Further experimental work now in progress is designed to elucidate the separate rôles which these long systems may have in the production of the crossed paralyses. It is thus possible, within certain limits, to alter at will the pattern of paralysis by varying the point at which the virus is introduced into the central nervous system. With this evidence for the propagation of the virus along the lines followed by definite fiber systems it seems possible to analyze the olfactory system for pathways which might be involved after intranasal inoculation.

9477 P

Experimental Poliomyelitis Without Paralysis.*

HOWARD A. HOWE AND ROBERT S. ECKE. (Introduced by P. H. Long.)

From the Department of Anatomy, Johns Hopkins Medical School.

Preliminary studies on a series of 13 Rhesus monkeys *(Macaca mulatta)* indicate that by section of the olfactory tracts it is possible to confine the activity of poliomyelitis virus after intranasal inoculation to the olfactory bulbs. Previous experiments (Brodie and Elvidge,[1] Schultz and Gebhardt[2]) have shown that monkeys in which both olfactory tracts and bulbs were destroyed did not develop paralysis after introduction of the virus into the nose. These findings, however, give no information regarding the ultimate fate of the virus after such a method of inoculation. The question remains as to whether the virus dies out immediately or may survive in the body—whether it can enter into any reactions with nonnervous tissue, or is capable of conferring any immunity upon its host although the typical picture of clinical poliomyelitis does not appear.

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* Supported by a grant from the President's Birthday Ball Commission for Infantile Paralysis Research.

[1] Brodie, M., and Elvidge, A. R., *Science*, 1934, **79**, 235.

[2] Schultz, E. W., and Gebhardt, L. P., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 728.

Eight animals were prepared with very careful sections of both olfactory tracts. This was done by an approach through the medial wall of the orbit, well proximal to the olfactory bulbs. The olfactory tracts were delivered through a small slit in the dura and were cut, in most cases without blood loss or damage to the underlying frontal cortex. Most important of all, there was no disturbance to the nervous tissue or blood vessels of the olfactory bulbs, so that the fibrils of the olfactory nerves presumably maintained their normal relations within the nasal mucous membrane. The operated animals and normal controls were inoculated intranasally on 2 successive days with one cc. of a 10-20% suspension of monkey cord containing MV virus. The controls showed a febrile reaction in 3-7 days with paralysis in 7-12 days from the date of inoculation. On the other hand the operated animals remained unparalyzed, although 6 of them developed a marked fever at the same time as the controls. This reaction subsided in 3-6 days' time and the animals were again apparently normal. A second inoculation in 3 animals produced another febrile attack, while the remainder were unaffected by the virus. In all, the third inoculation was unaccompanied by febrile manifestations.

In contrast was another group of 5 monkeys in which both olfactory bulbs had been removed. The operation was accomplished by cutting away the bridge of the nose and exposing the bulbs without directly entering or disturbing the nasal cavity. Thus visualized the bulbs were cleanly removed by suction with minimal damage to the adjacent frontal poles of the brain. All the animals so prepared showed neither paralysis nor temperature rise after the introduction of virus suspension into the nose, although the controls again pursued the course typical of poliomyelitis.

The experimental evidence thus points to the olfactory bulbs as the seat of a virus reaction which duplicates the early stages of clinical poliomyelitis. Sections of the olfactory bulbs from animals showing a febrile reaction show definite traces of virus invasion-leucocytic infiltrations, particularly around blood vessels, and destruction of mitral cells. It thus seems possible to produce poliomyelitis in the isolated olfactory bulbs without resulting paralysis. A minute histological study of the noses and nervous systems of the animals which have survived inoculation is now in progress. Also under investigation is the possibility that an immunity may be produced by this means.

9478

On the Stimulant Effect of Benzedrine Sulphate.

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Benzedrine sulphate has been reported as having a stimulant effect in certain types of patients with mental and physical fatigue and exhaustion. It has been found of value in arousing patients with narcolepsy,[1-3] chronic exhaustion,[4-6] and simple depression,[5] occasionally of value in depressed types of psychoneuroses[5, 7] but of little use in catatonic dementia praecox.[7, 8] Blackburn[9] believes that it raises the intelligence quotient in these patients. Experiments herein reported revealed that benzedrine sulphate does not stimulate spinal reflexes or skeletal muscle directly and that it is not depressant toward respiration as is epinephrine. This evidence supports the view that the stimulant action of benzedrine is entirely cerebral.

The time required for hind limb flexion after stimulating the pad of the foot with dilute (1-5000 to 1-1000) sulphuric acid in 20 brain-only pithed frogs was increased an average of 172% and in 85% of animals following the injection into the dorsal lymph sac of 0.05 mg. of benzedrine sulphate in 0.5 cc. of distilled water. The same dose of epinephrine hydrochloride or meta-synephrine hydrochloride had no consistent effect on the reflex time which on the average was slightly increased. Mean changes in the reflex time are shown in Fig. 1. In larger doses epinephrine also depressed the spinal reflex, an effect previously known but not generally recognized.[10] In relatively large doses benzedrine is thus even more depressant than epinephrine on the spinal reflexes of frogs.

The application of 0.001% benzedrine sulphate to the excised gastrocnemius muscle of 17 frogs produced a weaker and shorter contraction when the muscle was stimulated by a galvanic current.

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[1] Prinzmetal, M., and Bloomberg, W., *J. Am. Med. Assn.*, 1935, **105**, 2051.

[2] Ulrich, H., Trapp, C. E., and Vidoff, B., *Ann. Int. Med.*, 1936, **9**, 1213.

[3] Shapiro, M. J., *Minnesota Med.*, 1937, **20**, 28.

[4] Myerson, A., *Arch. Neurol. and Psychiat.*, 1936, **36**, 816.

[5] Wilbur, D. L., McLean, A. R., and Allen, E. V., *Proc. Staff Meet. Mayo Clinic*, 1937, **12**, 97.

[6] Nathanson, M. H., *J. Am. Med. Assn.*, 1937, **108**, 528.

[7] Davidoff, E., *Psychiat. Quart.*, 1936, **10**, 652.

[8] Carlisle, C. L., *Med. Bull. Veteran's Administration*, 1937, **13**, 224.

[9] Blackburn, J. M., *Lancet*, 1936, **2**, 1385.

[10] Solis-Cohen, S., and Githens, T. S., *Pharmacotherapeutics*, Appleton, London and New York, 1928, p. 1467.

A decrease in the height of contraction occurred in 94% of experiments, the average decrease was 39% and the standard deviation of the average decrease was 18%. A similar decrease occurred in 94% of corresponding experiments with epinephrine hydrochloride, the average decrease being 28%, with a standard deviation of 15%. These experiments demonstrated that benzedrine sulphate like epinephrine hydrochloride is depressant to skeletal muscle *in vitro*.

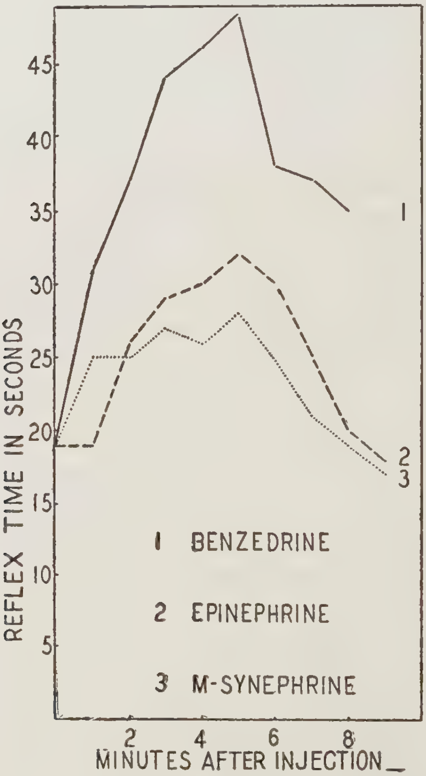

FIG. 1.

The effect of benzedrine, epinephrine, and meta-synephrine on the reflex time of frogs. Ordinate, reflex time in seconds; abscissa, minutes after injection.

Detrick, *et al.*,[11] obtained evidence that benzedrine may stimulate the respiratory center in experimental animals. This work was repeated in 8 rabbits anesthetized by urethane and ether with a Brodie tambour connected with the trachea. Fifteen to 20 intravenous injections of 0.05 to 1.0 mg. of benzedrine sulphate produced no significant change in respirations while corresponding doses of epinephrine hydrochloride depressed respirations in 95% of experiments and the average depression of the depth was 54%. Under corresponding conditions, therefore, benzedrine does not depress respirations of rabbits as does epinephrine.

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[11] Detrick, L. E., Millikan, R., Modern, F. S., and Thienes, C. H., *J. Pharm. and Exp. Therap.*, 1937, **60**, 56.

Conclusion. Benzedrine sulphate was found to depress spinal reflexes in brain-pithed frogs and to depress skeletal muscle *in vitro*. In doses at which epinephrine depresses respiration, benzedrine had no depressant effect.

9479 P

Nutritive Value of Lactalbumin versus Casein.*

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In this report a summary is given of the results of studies on growth, nitrogen storage, metabolism and composition of gains of rats on diets containing 8% and 12% lactalbumin and casein.

Growth Experiments. A total of 2 series of animals was used consisting of 3 groups. The rats of the same group were litter mates and of the same sex and weight, and all rats were confined in individual cages. One series of 9 animals was fed a diet containing 8% protein and another series was fed 12% protein. The animals were weighed every other day and accurate food consumption records were kept. The experimental period lasted 70 days. The average results of these growth experiments showed that the gain in body weight per gram of protein intake was 1.65 gm. for 3 animals fed a diet containing 8% casein, and 2.14 gm. for 6 animals on an 8% lactalbumin diet.

On a 12% level of protein these figures were 1.60 and 1.86 for casein and lactalbumin, respectively. Nitrogen storage was determined in the rats of the growth experiments described above. In each group a fourth rat, litter mate of the same sex and weight, was sacrificed at the beginning of the experiment and the carcass was analyzed for nitrogen. These rats served as controls. After the feeding period of 70 days all animals were sacrificed. The percentage of total food nitrogen stored on each ration was computed by subtracting the amount of nitrogen found in the controls killed at the beginning of the experiment from the amount of nitrogen found in the animals that had been on the ration for 70 days. The biological value of the protein was defined as the ratio (expressed in percentage) between the nitrogen stored in the body to the nitrogen intake during the feeding period.

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* Research paper No. 456, Journal Series, University of Arkansas, Fayetteville.

The biological value (according to this method) for casein was 26.3% (the average of the 3 casein-fed rats) and for lactalbumin was 37.5% (the average of 6 lactalbumin-fed rats) on the 8% level.

These figures were 29.2% for the casein rats and 36.1% for the lactalbumin rats on the 12% protein level.

Metabolism Experiments. Mitchell's[1] method was used for the determination of the biological value and a total of 28 rats was employed for these nitrogen balance experiments. Eight percent casein showed an average biological value of 69 for 12 rats; these figures were 84 for 8% lactalbumin (15 rats), 83 for casein and cystin (16 rats); 85 for lactalbumin and cystin (13 rats), 61 for 12% casein (6 rats), and 80 for 12% lactalbumin (6 rats).

The average true digestibility was 99 for 8% casein and 98 for 8% lactalbumin.

Another growth comparison of the values of both proteins at the 8% protein level was made by the modified paired feeding technique of Mitchell.[2] Nine pairs of rats were used. Litter mates and animals of the same weight and sex were employed in these pairs and the initial weight was about 52 gm. The food intakes of paired mates were equalized, but the lactalbumin protein ration was fed with varying amounts of a nitrogen-free ration. In this way equal gains were made and equal amounts of food were fed, but unequal amounts of nitrogen were taken in.

The 9 pairs of rats were killed at the end of about 60 days and the carcasses were analyzed for moisture, nitrogen, ash and fat in order to determine possible differences in the composition of the gain of the animals.

The results of the paired feeding experiments indicated that one gram of nitrogen from the lactalbumin ration was as effective in promoting the functions of maintenance and growth as an average of 1.33 gm. of nitrogen from the casein ration.

In similar experiments—employing 5 pairs of rats—it was found that one gram of nitrogen from the lactalbumin ration had the same nutritive effect as 1.08 gm. of nitrogen from the casein and cystin ration.

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[1] Mitchell, H. H., *Biol. Chem.*, 1924, **58**, 873.

[2] Mitchell, H. H., Burroughs, E. W., and Beadles, J. R., *J. Nutrition*, 1936, **11**, 257.

The carcasses of the lactalbumin-fed animals showed higher percentage of nitrogen storage than those of the casein-fed animals and it was calculated that one gram of food nitrogen from the lactalbumin ration promoted the same storage of nitrogen as 1.39 gm. of food nitrogen from the casein ration.

It was concluded that lactalbumin at an 8% and a 12% level of protein intake showed better growth and better nitrogen retention in rats than did casein at those levels, and it had the same or slightly better nutritive value as casein supplemented with cystin, and it was thus found to be a superior protein of high nutritive value for maintenance and growth.

9480

Effect of Various Salts on Hemolysis and Skin Necrosis Produced by Staphylococcus Toxin.

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Volk and Lipschutz[1] apparently were the first to study the action of hypertonic salt solutions on staphylococcus hemotoxin. The salts used were NaCl, Na_2SO_4, $MgSO_4(NH_4)_2$, SO_4 and $BaCl_2$. It was their opinion that staphylococcus toxin was not destroyed but that the hemolytic action was inhibited in the presence of hypertonic concentrations of the neutral salts with one exception and that was with $BaCl_2$.

Recently one of us has studied the effect of NaCl on the hemolytic and skin necrotizing factors in staphylococcus toxin.[2, 3] It was found that a hypertonic solution of NaCl inhibited the degree and rate of lysis of rabbits' red blood cells. When a 5% concentration of NaCl was injected intradermally in rabbits prior to the intradermal injection of staphylococcus toxin skin necrosis was inhibited at the site of toxin injection.

The above observations suggested a further study of the effect of

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[1] Volk, R., and Lipschutz, B., *Wien. klin. Wchnschr.*, 1903, **16**, 1394.

[2] Rigdon, R. R., *J. Infect. Dis.*, 1937, **60**, 25.

[3] Rigdon, R. H., *Arch. Path.*, 1936, **22**, 763.

TABLE I.

Effect of Various Salts on Hemolysis Produced by Staphylococcus Toxin.*

	Hemolysis					
Salts	Salt + R.B.C.			Salt + R.B.C. and Staphylococcus Toxin		
Concentration	M/1	M/2	M/4	M/1	M/2	M/4
NH_4Cl	4	3	—	4	4	—
NH_4Br	4	2	—	4	4	—
NH_4F	0	2	4	0	3	4
NH_4NO_3	4	3	—	4	4	—
$(NH_4)_2SO_4$	±	0	3	3	4	4
NH_4CnS	4	4	4	—	—	—
$(NH_4)_2HPO_4$	0	3	2	4	4	4
$(NH_4)_3$Cit	P	P	P	—	—	—
$(NH_4)_2$Ox	4	4	4	—	—	—
NH_4Lact.	4	4	4	—	—	—
NH_4Acet.	4	4	4	—	—	—
LiCl	0	0	0	3	4	4
LiBr	1	0	0	2	4	4
$LiNO_3$	±	0	0	±	3	4
Li_2SO_4	0	0	0	±	3	3
Li_3Cit	±	±	±	±	0	1
Li_2Ox	0	0	1	2	4	4
LiLact	0	0	0	1	4	4
LiAcet	0	0	0	1	3	3
NaCl	0	0	0	1	4	4
NaI	1	0	0	0	3	4
NaBr	0	0	0	0	3	4
NaF	4	4	4	—	—	—
$NaNO_3$	0	0	0	0	3	4
Na_2SO_4	0	0	0	3	3	4
NaCNS	4	0	0	1	2	4
Na_2HPO_4	4	4	4	—	—	—
Na_3Cit	±	±	±	4	4	4
Na_2Ox	i	i	±	i	i	4
NaLact	0	1	0	4	3	4
NaAcet	0	0	0	3	3	3
KCl	0	0	0	3	4	4
KI	3	0	0	0	2	4
KBr	0	0	0	0	4	4
KF	1	±	0	3	4	4
KNO_3	0	0	0	0	3	4
K_2SO_4	i	0	0	i	3	4
KCNS	4	3	1	4	4	4
K_2HPO_4	0	0	0	3	3	4
K_3Cit	±	±	0	4	4	3
K_2Ox	0	0	0	3	3	4
KLact	0	0	0	3	3	4
KAcet	4	1	1	—	4	4
$MgCl_2$	0	0	0	0	1	4
$MgBr_2$	4	4	3	4	4	3
$Mg(NO_3)_2$	3	±	0	4	0	±
$MgSO_4$	±	0	0	±	1	2
$MgAcet_2$	1	1	1	1	2	3
$CaCl_2$	2	2	1	2	2	2
$Ca(NO_3)_2$	2	1	±	P	P	P
$SrCl_2$	3	±	0	2	1	2
$Sr(NO_3)_2$	P	2	±	P	P	P
$BaCl_2$	±	0	0	±	±	1
$Ba(NO_3)_2$	i	i	0	i	i	1

*1, 2, 3, and 4, degree of lysis.
P, heavy precipitate.
i, insoluble.
—, not done.

various salts on the hemolytic and skin necrotizing factors in staphylococcus toxin. The salts studied are given in Table I. The toxin preparation is the same as previously used.[2, 3] Washed rabbits' red cells were added to M/1, M/2, and M/4 solutions of the different salts to make a 1% suspension in a 2 cc. volume. The least amount of toxin which would completely hemolyse this concentration of cells suspended in 2.0 cc. of physiological saline was the amount of toxin used in all the tests. The toxin was added not less than 10 minutes after the cells were placed in the salt solutions. The titrations were read after standing at room temperature over night. The control titrations were run without toxin. The pH of all the salt solutions was determined.

Table I shows the effect on the blood cells of the various salts alone and in the presence of the hemolyzing unit of staphylococcus toxin.

From this table it can be seen that certain salts are hemolytic, while other salts are either slightly hemolytic or entirely non-hemolytic. Furthermore NH_4F, LiBr, $LiNO_3$, Li_2SO_4, Li_3 citrate, Li lactate, Li acetate, NaCl, NaI, NaBr, $NaNO_3$, NaCNS, Na acetate, KI, KBr, KNO_3, inhibit the hemolytic action of staphylococcus toxin. Of these salts the halogens and the nitrates show the strongest inhibiting effect.

The methods used to study skin necrosis are the same as given previously.[3] All the salts used in the hemolytic study were injected into rabbits intradermally in 0.5 to 2.0 cc. amounts.

A majority of these salts produced some skin necrosis in either M/1, M/2 or M/4 concentration. Solutions of NH_4Cl, LiCl, NaCl, K_2SO_4, Na lactate, Na acetate, and $MgSO_4$ failed to produce necrosis in the skin in the concentrations used. When either LiCl or $MgSO_4$ in 2 cc. quantities was injected intradermally immediately to 15 minutes before the injection of toxin no skin necrosis occurred at the immediate site of toxin injection. An equal volume of K_2Ox similarly injected into rabbits increased the extent of the skin necrosis.

Several of the results of the above experiments are particularly interesting. First, the effect of certain salts on the hemolysin and the skin necrotizing factors in staphylococcus toxin should be noted. This is strikingly illustrated by LiCl and $MgSO_4$. The former salt inhibits necrosis at the site of injection but has little or no effect on inhibiting hemolysis. The latter, $MgSO_4$, shows a marked suppression of both the hemolytic and skin necrotizing factors. Secondly, the effect of certain salts alone on rabbits' red blood cells and skin are interesting, for example, the oxalates of Li, Na, K, have little or no hemolyzing power but cause extensive necrosis of the skin. Thirdly, the precipitation of staphylococcus toxin by certain of these salts, such as $(NH_4)_2SO_4$, the salts of Ba and of Sr, and more particularly the precipitation by calcium chloride and the recovery of the toxin with K_2Ox, which will be subsequently reported, are worthy of note.

9481

Attempts to Demonstrate Virus-Neutralizing Substances in Saliva and Serum from Mumps Immunes.*

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Active immunity to experimental mumps parotitis in *Macacus rhesus* has been studied by Johnson and Goodpasture,[1] with the following conclusions: "The only reliable experimental method at present of inducing active immunity to mumps in monkeys is by causing a unilateral or bilateral clinical or subclinical specific parotitis by intraparotid inoculation." They failed, except rarely, to demonstate any passive immunity to mumps in monkeys previously injected with the serum of persons immune to this disease, or any marked virus-neutralizing action of human convalescent serum.

As a further step toward understanding the mechanism of active immunity to experimental mumps, the present study has been made concerning the virus-neutralizing properties of human and monkey saliva and of monkey serum.

For this purpose it was decided to inject a number of normal *Macacus rhesus* monkeys by the usual transductal route with variously treated mixtures of mumps virus (saline suspensions of infected glands) and saliva (or serum) from immune or normal animals and human beings. One parotid would receive the immune substance, the other the normal, and the presence or absence of virus-neutralizing substances would be shown by the presence or absence of or difference in the mumps "takes" on the 2 sides, with respect to time of onset and size of gland.

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* Aided by grants from the Division of Medical Sciences, Rockefeller Foundation, and the Josiah Macy, Jr., Foundation.

† National Research Council Fellow in Pathology.

[1] Johnson, C. D., and Goodpasture, E. W., *Am. J. Hygiene,* 1936, **23**, 329.

Several monkeys were actively immunized by unilateral of bilateral injections, into the parotid duct, of centrifuged saline suspensions of the parotid glands of monkeys in the active stage of experimental mumps. The virus used had been recently obtained from the saliva of a boy in the first day of clinical mumps, and had undergone several typical monkey passages, with characteristic clinical signs and gross and microscopic changes in the glands.

When the inoculated animal developed, on the 6th to 8th day, fever, swelling of the parotid gland or glands, with or without edema of the overlying tissues, and enlargement of the adjacent lymph-nodes, it was assumed, on the basis of the constantly positive results obtained by Johnson and Goodpasture, that it had mumps, and would subsequently be immune. It did not, therefore, seem necessary to test each animal's immunity by reinoculation at a later time.

Saliva was obtained from immune and from normal monkeys under nembutal anesthesia (Nembutal 30 mg. per kilo, intraperitoneal), by administering pilocarpine hydrochloride (one mg. per kilo) and collecting the fluid which drooled from the mouth. Attempts to obtain the parotid secretion by itself, through suction cups and cannulas, were unsuccessful. The saliva was centrifuged to get rid of solid particles, and certain samples were then filtered through a Berkefeld V candle. Others were used unfiltered.

The saliva-virus mixtures consisted of equal parts of saliva and of a 20% saline suspension of the infectious glands, giving a final tissue percentage of 10%, which concentration had been consistently found sufficient by itself in 2 cc. amounts, to cause mumps. The dose of the virus-saliva mixtures was 3.5 cc.

Human saliva, from immunes and normals, was employed in the same manner.

Except in 2 experiments, in which the mixed inoculums were placed in the icebox overnight, and one in which the inoculum was heated to 55°C. for ½ hour, all the virus-saliva mixtures were held at 37°C. for 2 hours before injection, to give presumably enough time for antibody-virus combination.

Monkey serums were obtained from either the saphenous vein or the heart, mixed in equal parts with virus suspensions, and heated, as above, at 37°C. for 2 hours.

Both salivas and serums of immune monkeys were taken between one and 2 months after inoculation with mumps. Human salivas were taken one month and 2 months after the onset of the disease.

Whether or not a test animal had a "take" was determined entirely on clinical grounds, by swelling, with or without edema, of one or the other parotid gland. The animals were not killed, both for reasons of economy and because it was necessary to observe them for longer than the usual period to detect possible delayed "takes".

Of 12 monkeys receiving either human or monkey immune saliva and virus on one side and normal saliva and virus on the other, only 2 showed a better "take" in the normal saliva parotid than in the immune saliva parotid. In one, which had received mixtures whose saliva had been heated to 55°C. for ½ hour, there was no "take" on the immune-injected side, and a moderate enlargement of the normal-injected side. In the other both glands showed some swelling, but it was much more marked on the side which had had the normal saliva mixture. A third monkey showed a slight delay in the onset of swelling on the immune side, but the ultimate maximum swelling was the same in both glands.

The rest of these 12 monkeys either showed the greater enlargement on the immune-saliva side (in 2 cases) or showed no distinct difference between the 2 glands.

Of the 4 monkeys injected with immune serum and virus in one parotid and with normal serum and virus in the other, one had equal enlargement on the 2 sides, one showed no swelling one either side, a third had a small "take" only on the immune-serum side, and the fourth had just the opposite, a "take" only on the normal side.

One monkey was used as a control. It received normal and immune monkey salivas without virus, and was negative both clinically and microscopically.

The results of these experiments are obviously indecisive and fail to demonstrate the presence of any antiviral substance in either the saliva or the serum of monkeys, or in the saliva of human beings recently recovered from mumps. The variations in reaction with the different mixed inoculums are no greater than might occur accidentally, especially if one considers the summer decline in mumps "takes" which has been repeatedly observed, and which may have affected a few of the later experiments in this series.

One comes to the same conclusion as Johnson and Goodpasture, that the study of immunity to mumps requires a more delicate test of the presence of antibodies than is provided by intraparotid inoculations. If there were some other, less direct way, as yet undiscovered, of inducing the specific parotitis, which would give a more delicate balance between the infecting agency and the immunizing substance, significant experiments might be performed. It is also most important to develop a way of measuring and standardizing the virus content of the tissues used for inoculums. Without having a minimal infective dose, it is impossible to detect small amounts of immune substances in fluids or tissues.

Summary. 1. The parotid glands of monkeys were injected with various mixtures of mumps-virus-containing gland suspensions and of saliva or serum of mumps-immune and normal monkeys, and saliva from immune and normal human beings. 2. The results of these injections give no evidence for the presence of any considerable amount of antiviral substance in either serum or saliva. 3. More delicate and accurate techniques are needed to demonstrate the nature of antiviral immunity to mumps.

9482

Attempted Chemical Isolation of the Virus of Poliomyelitis.

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The demonstration of the chemical nature of the tobacco mosaic virus by Vinson and Petre,[1] its recent isolation as a crystalline protein by Stanley,[2] and the experiments of Howitt[3] indicating that the virus of poliomyelitis may be also a chemical entity, have led us to employ Stanley's procedure in an attempted isolation of the virus of poliomyelitis in the form of a crystalline chemical substance.

This attempt was not successful but, because the results are of interest in that they afford added evidence for the theory of the chemical nature of viruses, they are briefly reported herewith.

One hundred grams of spinal cords from monkeys infected with the M.V. strain and developing typical paralysis in from 7 to 11 days after intracerebral inoculation, were finely ground with sand and extracted overnight in the refrigerator with 500 cc. of a solution of disodium hydrogen phosphate at pH 8. The thoroughly mixed suspension was poured onto a fine wire screen and the retained precipitate again extracted for 24 hours with 500 cc. of the phosphate solution, poured through the screen and the combined milky suspensions filtered by gravity through coarse fluted filter paper. A monkey inoculated with 0.5 cc. of this filtrate developed typical paralysis in 9 days.

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[1] Vinson, C. G., and Petre, A. W., *Bot. Gaz.*, 1929, **87**, 14.

[2] Stanley, W. M., *Science News Serv.*, 1935, **81**, 644.

[3] Howitt, B., Proc. Soc. Exp. Biol. and Med., 1930, **28**, 158.

The filtrate was weighed and sufficient solid ammonium sulfate added to bring the final concentration to 30% by weight. The precipitate of crude globulin was separated by gravity filtration and extracted twice with 500 cc. portions of phosphate solution at pH 8. The combined filtered extracts were again precipitated with ammonium sulfate at 30% concentration and again extracted with two 500 cc. portions of phosphate solution.

The combined filtered extracts were now precipitated with ammonium sulfate at 20% concentration and the globulin dialyzed against distilled water until free from sulfate.

This purified globulin was then extracted for 24 hours with 500 cc. of phosphate solution at pH 8, filtered with suction through a layer of celite, and dilute acetic acid added to the slightly opalescent filtrate to the point of apparent maximum precipitation (about pH 4). Since microscopic examination of the suspension after standing overnight in the refrigerator gave no evidence of crystal formation, the precipitate was filtered off, mixed with a small amount of celite and again filtered through a pad of celite. The celite filter cake was extracted 3 times with 100 cc. portions of phosphate solution and the combined extracts filtered through a layer of celite. The clear filtrate was again treated with dilute acetic acid to the isoelectric point but despite the apparent purity of this filtrate no crystals were observed in the precipitate. The latter was therefore filtered off, dissolved in phosphate solution at pH 7.5 and a monkey inoculated with 0.5 cc. of the clear solution. Typical flaccid paralysis developed in 11 days.

The final globulin solution appeared quite pure, had a protein content of 0.01%, was free from salts present in the original cord, and precipitated fairly sharply at pH 4.0. To assume an organism capable of withstanding the foregoing treatment presupposes one of unusual vitality, possibly encapsulated. On the other hand, the reactions involved are not inconsistent with what may be expected of a pure globulin and, though very intimate association of an organism with this protein has not been excluded, the above series of reactions point strongly to the etiologic agent in poliomyelitis being a chemical (protein) agent or closely associated with it.

9483

Difficulties in the Use of Brachycardia Method of Assaying Vitamin B_1.

ELIZABETH CHANT ROBERTSON AND M. ELIZABETH DOYLE.
(Introduced by F. F. Tisdall.)

From the Department of Pediatrics, University of Toronto, and the Hospital for Sick Children, Toronto.†

Using the brachycardia method as described by Harris and Leong,[1] we have attempted to assay vitamin B_1 hydrochloride (Merck),* and the international standard of vitamin B_1. Young piebald rats from our own rat colony, when 45 to 50 gm. in weight, were put on a vitamin B_1-free diet made up of crude casein 18%, McCollum's Salt Mixture (No. 51) 4.5%, sugar 61.5%, cod liver oil 1%, and autoclaved yeast 15%. The latter was strongly alkalinized, autoclaved at 15 lb. pressure in thin layers for 5 hours, and then neutralized and ground. The rats were all kept in wire cages, having meshes ⅝ in. square, in order to prevent possible coprophagy. After about 12 days on the diet, the rats were weighed daily. When the body weights were constant, electrocardiograph records, using a standard Cambridge electrocardiograph, were taken each day. When the heart rate came down to 370 to 390 per minute, the rat was given a single dose of vitamin B_1. A solution of the vitamin B_1 hydrochloride was made up daily in distilled water, and the international standard was weighed dry. The dose was mixed with a little autoclaved yeast and then fed to the rat. At least 5 rats received each dose, and they usually ate it within one to 1½ hours. If they took longer than 4 hours, the results were often unsatisfactory. Daily electrocardiograms were taken on each rat at about the same time each morning. The rats were held gently by hand while the records were being made and very rarely struggled or squealed during the process. Great care was taken not to hold the skin tightly, and there was no possibility of asphyxia complicating the results. At least 40 beats were counted to get the heart rate, and duplicate readings were frequently made on the tracings.

Even in one tracing, it was often found that the rate was at least 40 per minute faster in one part than another, and 2 tracings from one rat taken within half an hour sometimes showed differences of 100 beats a minute.

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† Under the direction of Alan Brown, M.D., F.R.C.P.(C).

[1] Harris, L. J., and Leong, P. C., *Lancet*, 1936, **1**, 886; *Biochem. J.*, 1937, **31**, 672. See also Baker, A. Z., and Wright, M. D., *Biochem. J.*, 1935, **29**, 1803.

* We are indebted to Merck & Co. for supplies of this material.

We have not been able to get graded responses with graded doses. For example, when the following doses of vitamin B_1 hydrochloride were fed, the irregular results shown in Table I were obtained.

TABLE I.

Experiment	Dose	Interval before back to original rate, days
1	3γ	4
	6γ	2.6
	9γ	3.6
	12γ	3.2
2	3γ	2.6
	6γ	6
	9γ	4.5

Anomalous results such as the following were not infrequently encountered. When the rate was 389 per minute, the rat was fed 6 γ. On the next 5 days the rates were 480, 345, 389, 476, 326.

Several variations in the technique were tried. For instance, smaller rats were used which weighed about 30 gm. when they were put on the deficient diet. Also the vitamin B_1 was given when the heart rates were from 400 to 420 per minute. Also larger doses ranging from 12 γ to 30 γ, and from 2.5 to 7.5 I. U. were used. None of these alterations improved the results.

In all, we have studied the responses of 179 rats to single doses of vitamin B_1, and in our hands this method gave inconsistent results.

9484

Dietary Calcium and pH of the Lower Intestine.*

ELIZABETH CHANT ROBERTSON. (Introduced by F. F. Tisdall.)
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Shortly after weaning, litters of young rats were divided into 5 groups and fed the 5 following diets for 7 to 11 weeks, when they were killed and the pH at various levels of their intestinal tract was determined, using the B.D.H. capillator. The capillator was checked with a potentiometer and in the pH range of 5.5 to 7.5, they checked very well.

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* An excerpt from a thesis submitted in conformity with the requirements for the degree of Doctor of Philosophy in the University of Toronto.

† Under the direction of Alan Brown, M.D., F.R.C.P.(C).

All the diets contained corn starch (60.5%-64%), casein (18%), Crisco (10%), dried brewer's yeast (6%), and cod liver oil (2%). This diet with no additions was the mineral deficient diet. In the second diet, 1% K_2CO_3 was added to the mineral deficient diet; in the third, 1% $CaCO_3$ was added; in the fourth, 1% K_2CO_3 and 1% $CaCO_3$ were added; and in the fifth, 3.5% of Hawk and Oser's[1] salt mixture was added and this contains approximately as much Ca and K as 1% $CaCO_3$ and 1% K_2CO_3. The fifth diet was known as the adequate diet.

The pH readings in the stomach, duodenum and jejunum were practically the same regardless of the diet fed. In the rats fed the diets low in calcium (that is, those fed the mineral deficient or the mineral deficient diet + 1% K_2CO_3) there was a slightly more acid reaction (pH 6.5-6.6) in the lower ileum than in those fed the other diets, which were rich in calcium, where the pH was from 6.9-7.0. The former rats had abnormally large amounts of fecal material in their lower ileums.[2] The reactions in the cecums of the rats on the calcium poor diets varied from 5.9 to 6.1, as compared with 7.0 to 7.1 in the rats on the calcium rich diets. The readings in the colons were 6.0 with the calcium poor and 7.0 with the calcium rich diet. These figures are the averages of determinations made on at least 25 different rats, and in many cases (*e. g.*, those on the adequate and mineral deficient diets) 70 to 100 rats were used. These results are summarized in Table I.

TABLE I.
pH in Lower Intestines of Rats Fed Various Diets for 7-11 Weeks.

	Min. Defic. Diet	Min. Defic. Diet + 1% K_2CO_3	Min. Defic. Diet + 1% $CaCO_3$	Min. Defic. Diet + 1% K_2CO_3 + 1% $CaCO_3$	Adequate Diet
Colon	6.0	6.0	7.0	7.0	7.0
Cecum	5.9	6.1	7.0	7.0	7.0
Ileum	6.6	6.5	6.9	7.0	7.0

The standard deviations of the readings calculated according to the method of Banister[3] varied from 0.2 to 0.3. At each of these 3 levels in the intestinal tract the differences in pH between the rats fed the calcium poor and the calcium rich diets were statistically significant. The rats fed the calcium poor diets showed marked stasis and dilatation in the large intestines, whereas those fed the calcium rich did not show this.[2]

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[1] Hawk, P. B., and Oser, B. L., *Science*, 1931, **74**, 369.

[2] Robertson, E. C., *Am. J. Dis. Child.*, 1937, **53**, 500.

[3] Banister, H., *Elementary Applications of Statistical Method*, Blackie, London, 1929, p. 24.

From these results it appeared that the calcium in the diet was the factor responsible for keeping the pH of the lower intestine around 7.0. To test out this hypothesis, diets were made up which were adequate except for calcium, adequate except for potassium, and adequate except for calcium and potassium, by means of leaving out the Ca and/or K salts from the salt mixture.

Young rats were fed these diets and similar controls were fed the mineral deficient and the adequate diet. After 7 to 11 weeks, the pH determinations were made as before. The results obtained are shown in Table II, each figure being the average from at least 20 rats.

TABLE II.
pH in Lower Intestines of Rats Fed Various Diets for 7-11 Weeks.

	Min. Defic. Diet	Adeq. except K and Ca	Adeq. except Ca	Adeq. except K	Adequate Diet
Colon	5.9	6.0	6.2	7.1	7.2
Cecum	6.0	6.1	6.3	7.1	7.3
Ileum	6.7	6.7	6.8	7.1	7.0

When the rats were fed the diets low in calcium (first 3 diets) the pH values of the large intestine varied from 5.9 to 6.3 and these rats had intestinal stasis. On the other hand,when the diets were rich in calcium (last 2 diets), the pH in the large intestine varied from 7.1 to 7.3 and these rats did not have intestinal stasis.

The stasis apparently occurs only when the calcium intake is low, and the acid reaction in the large intestine occurs when there is stasis. The feces of the rats fed the adequate diet contained about 6.2% Ca, those of rats on the mineral deficient diet contained about 0.18% Ca. In a few rats on the deficient diet the excretion of calcium was trebled by means of feeding 2 grains of desiccated thyroid (Parke, Davis & Company)[4] daily, but no constant change in the reaction of the large intestine occurred.

By means of a Petroff-Hausser bacterial counter, counts were made of the bacteria in the cecums of rats fed the adequate and the mineral deficient diets. It was consistently found that there were many more organisms in the deficiently fed (14.5×10^{10} per gram, on the average), than in the adequately fed animals (average 4.7×10^{10}). Spread plates of dilutions of the cecal contents gave similar results. Possibly the greater acidity is due to the biochemical activity of the more numerous bacteria in the mineral deficient animals. Kjeldahl determinations showed that there was 10.2% protein in the cecal material in the deficiently fed as compared with 6.8% in the adequate controls. The higher protein might favor bacterial growth.

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[4] Pugsley, L. I., and Anderson, E., *Biochem. J.*, 1934, **28**, 754.

Conclusions. Young rats fed diets low in calcium have a pH of about 6.0 in their large intestines. Controls fed the corresponding adequate diet have a pH of about 7.0.

9485

Additional Sources of Androgens.

M. D. Kritzer and B. Cunningham. (Introduced by W. A. Perlzweig.)

From the Department of Zoology, Duke University.

Stallion Urine. It is rather surprising that the occurrence of androgens in stallion urine has not been reported, or if it has that the record has not found its way into the literature of the subject, since stallion urine has been studied in relation to estrogenic content. For this reason we have made assays upon stallion (Percheron) urine and report them here. The occurrence of an androgenic substance in stallion urine was demonstrated in our laboratories by Mr. Norman Nathenson in 1936, but his assays were erratic due to massive doses. The studies were repeated by us during the fall of 1936, smaller doses being used and fairly concordant assays being obtained. The urine was acidified to Congo red with sulfuric acid and allowed to stand 2-3 days. It was then extracted with benzene, evaporated to dryness, residue taken up in ether, washed with 10% NaOH solution, dissolved in olive oil and assayed by the capon comb method. The assays showed a bird unit in (1) 548 cc., (2) 570 cc., (3) 562 cc.; an average of 560 cc. per bird unit. (A bird unit is taken herein to be the amount of each of 5 daily injections required to produce a total of 5 mm. growth in length plus height as measured on the 6th day.)

Dog Urine. Dog urine was collected from a male German police dog; the same animal serving as a source for 2 experiments with an interval of about a year intervening. The urine was extracted in the same manner as that of the stallion. The first sample was assayed on 4 birds, the latter on 2. The amount of urine necessary for a bird unit was as follows: (1) 790 cc., (2) 500 cc., (3) 650 cc., (4) 415 cc., (5) 920 cc., (6) 650 cc. Average 652 cc. per bird unit.

Chicken Feces. For these studies rooster and capon feces were used. In each case 630 gm. of fresh feces were shaken with water acidified to 1% with sulfuric acid and allowed to stand several days before filtering. The filtrate was extracted in the same manner as the stallion urine.

The rooster feces extract was assayed on 3 birds and the amount of original feces required to give a bird unit was found to be (1) 84 gm., (2) 78 gm., (3) 70 gm. Average 77 gm. per bird unit.

The capon feces showed no growth effect even when the equivalent of 310 gm. of feces was injected subcutaneously into a single bird. When injected into the base of the comb the equivalent of 250 gm. produced growth of less than 3 mm. Since injection into the base of the comb is about 20 times as effective as intramuscular injection[1] it would appear that there is about one bird unit in 1000 gm. of the capon feces. The small amount of androgens in capon feces is surprising in view of the fact that it is easily demonstrated in hen feces.[2] This divergence may be explained by the method of extraction, which in one case was specifically for the female hormone and in the other for the male hormone.

9486 P

A Vasopressor Phenomenon Following Intra-arterial Administration of Hypertonic Solutions.

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(Introduced by Vincent du Vigneaud.)

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The behavior of hypertonic solutions in the blood stream has been investigated both in normal[1-5] and in shocked animals.[6, 7] In all instances there occurs a temporary blood dilution which is diminished in hemorrhagic shock[8] and increased in dehydration.[8] During an investigation of the local fluid loss resulting from trauma, a pressure phenomenon was noted following the injection of hypertonic electrolyte solutions into the arterial system. This study constitutes a preliminary report of this phenomenon with particular reference to its nature and basic mechanism.

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[1] Fussganger, *Med. Chem. (I. G. Farbenindustrie)*, Leverkusen, 1934, p. 194.

[2] Domm, Gustavson, and Juhn, in Allen, *Sex and Internal Secretions*, p. 626.

[1] Moritz, F., *Deutsch. Arch. Klin. Med.*, 1887, **41**, 395.

[2] Weed, L. H., and McKibben, P. S., *Am. J. Physiol.*, 1919, **48**, 512, 531.

[3] Keith, N. M., *Am. J. Physiol.*, 1924, **68**, 80.

[4] Adolph, E. F., and Lepore, M. J., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 963.

[5] Davis, H. A., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 242.

[6] Erlanger, J., and Gasser, H. S., *Ann. Surg.*, 1919, **69**, 389.

[7] Davis, H. A., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 21.

Dogs weighing from 10 to 15 kg. were used. Nembutal was the basic anesthetic. The right and left femoral arteries and veins were exposed and, also, in several experiments, the brachial arteries. The arterial blood pressure was recorded from the carotid artery by the usual methods. The following solutions were utilized: (1) 25% sodium chloride solution; (2) 50% glucose solution; (3) 30% gum acacia dissolved in 0.9% sodium chloride solution; (4) 50% sucrose solution. The quantity of solution used in each experiment was 5 to 10 cc. A series of 20 experiments was performed.

The intraärterial injection of hypertonic solutions results in an elevation of the systolic blood pressure from 10 to 50 mm. Hg. above the initial level. The duration of this pressor response is from 1 to 3 hours or even longer. The effectiveness of the solutions varies. Sodium chloride solutions produced the most prolonged elevation of blood pressure, whereas solutions of gum acacia had little, if any, result. Control solutions of isotonic sodium chloride did not increase the blood pressure. Certain other local and general effects were noted during and following the administration of these hypertonic solutions. During the injection, the muscles of the extremity, whose arterial supply was being used, became tense and frequently contracted strongly. Weight experiments of the affected area before and after injection revealed a diminution in weight, which, it is suggested, results from a loss of tissue fluid by a process of osmosis. The respirations are at first greatly increased in rate and depth, but later become slow and regular in rhythm. The intravenous injection, in contrast to the intraärterial, produces a depression of the blood pressure with a return to the initial level within 3 to 5 minutes. If the blood pressure has been lowered previously from any cause, the depression following intravenous administration of the solution may reach a critical level.

Total section of the spinal cord in the region of the 12th dorsal segment completely abolished the pressor reaction. Following bilateral adrenalectomy, the pressor response to intraärterial hypertonic solutions took the form of a more gradual elevation of blood pressure which, however, was maintained. Division of the sciatic and femoral nerves of the affected area did not have any significant effect upon the pressor phenomenon. The vasodepressor effect of hypertonic solutions administered by the venous route was also studied. Bilateral vagotomy did not prevent the fall of blood pressure, suggesting the presence of other factors in this reaction. This investigation has not yet disclosed the nature of the factors involved.

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[8] Davis, H. A., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 210.

The phenomenon of vasopression following the administration of hypertonic solutions of sodium chloride by the intraärterial route can be attributed to a stimulation of the peripheral nerve endings in the involved area with a resultant generalized vasoconstriction effected by the central nervous system. The degree of stimulation of the peripheral nerves of the extremity varies directly with the amount and hypertonicity of the solution used. In view of this fact, the demonstrable relationship between water metabolism and chronaxie of nerve[9] is interesting. The release of protein-containing tissue fluid from the extremity, resulting in an increase in the circulating blood volume, is of lesser importance, in spite of recorded losses in weight up to 100 gm. of areas studied in several experiments.

Summary. A prolonged elevation of blood pressure occurs after the intraärterial administration of hypertonic solutions of sodium chloride. This pressor effect appears to be predominantly nervous in origin, inasmuch as it disappears after section of the spinal cord.

9487

Treatment of Pneumococcal Infections in Rabbits with Sulfanilamide.

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A number of studies have been made to show the curative effect of sulfanilamide in rats, mice, guinea pigs and rabbits, infected with pneumococci by various routes. Because of the difference of opinion concerning the efficacy of the drug in the treatment of infections caused by this microörganism, it was considered timely to investigate further its effects on controlled pneumococcal infection. For this purpose, infection was induced in rabbits by injecting Type I pneumococci endermally according to the method of Goodner,[1] which permits accurate observations of the fever, bacteremia and of the lesion produced locally.

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[9] Achelis, J. D., *Pflüger's Arch.*, 1930, **226**, 212.

The inoculum consisted of 0.1 cc. of a 1:1000 dilution of an 18-hour blood-broth culture injected endermally in the shaved skin of the abdomen. Rectal temperature was recorded twice daily and blood cultures were made daily until the animal died or recovered. In each of 6 sets of experiments, 4 rabbits were studied; 2 animals were treated and 2 served as controls.

For treatment, sulfanilamide (para-amino-benzene-sulphonamide) was chosen in preference to prontosil because previous experience had shown that the latter drug exerted little influence on the course of pneumococcal infection in rabbits. The limited solubility of prontosil requires the injection of larger volumes of fluid than is practical. The sulfanilamide (supplied by E. R. Squibb & Sons) was prepared in 1% solution and was administered by means of a stomach-tube—100 cc. twice in 24 hours. This daily dose is equivalent to a total of 2.0 gm. of sulfanilamide and is well within the limits of that tolerated by rabbits weighing 2 kg. According to Raiziss, Severac and Moetsch,[2] a dose of 1.5 gm. per kilo of body weight was tolerated by 94% of the rabbits, while a dose of 2 gm. per kilo was tolerated by 50% of the animals.

The strain of Type I pneumococcus used proved to be exceptionally virulent for rabbits. All of the untreated control animals died, usually between the second and fourth day after inoculation. Bacteremia with high fever was present in each case. The local lesion about the point of inoculation consisted of a swollen, indurated area about 30 mm. in diameter with erythema extending to 80 or 90 mm.

In the various series, treatment was started at different intervals after injection of the pneumococci—6, 8, 18, 20, 30 and 48 hours after inoculation, respectively. In the last series (48 hours) 2 rabbits were dead at the time treatment was started on the 2 remaining animals. Both of the latter succumbed, although one lived 9 days. At the time of death, the blood culture showed no growth of any organisms and the local lesion had disappeared entirely. Death may have been due to the toxic effects of the drug.

The results of treatment of 10 rabbits in the other 5 experiments were more encouraging—7 of the animals survived. The blood cultures of *all* the treated animals became negative in 3 to 5 days, their temperatures dropped to normal and the local lesions promptly healed. Yet 3 died, possibly because of the toxicity of the drug.

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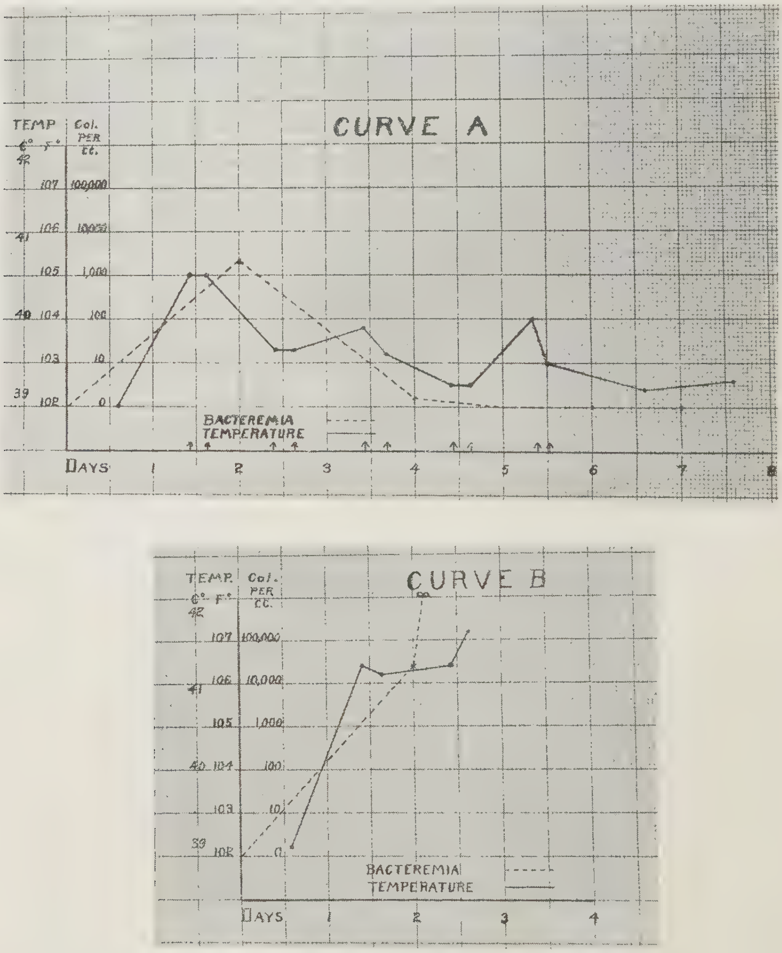
[1] Goodner, K., *J. Exp. Med.*, 1928, **48**, 413.

[2] Raiziss, G. W., Severac, M., and Moetsch, J. C., *J. Chemotherapy*, 1937, **14**, 1.

The set of rabbits in which treatment was started 20 hours after inoculation may be described as typical of the results obtained in the other experiments. At the time treatment was instituted the temperatures of the 4 rabbits ranged between 105.0° and 106.4°F. and blood cultures revealed many pneumococci—the lowest count being 3200 colonies per cc. of blood while the plates made from 2 of the rabbits were uncountable. In addition to this well established bac-



The temperature and the number of pneumococci per cc. of blood at various intervals in a rabbit (A) treated with sulfanilamide, as indicated by arrows, and in an untreated control (B).

teremia, each animal had a local lesion (80 to 90 mm. in diameter) about the point of inoculation which presented an intense erythema and marked induration. Two of the rabbits were given 100 cc. of a 1% solution of sulfanilamide by mouth twice each day. After 3 days, the blood cultures showed no growth and at the end of 5 days the temperature had returned to normal and the local lesions had healed. Each rabbit received a total of 10 gm. of the drug during the period of treatment. It should be noted that the dose used was far in excess of that recommended in human therapy.

Both of the untreated rabbits died in 2 days. There was no reduction in the bacteremia or extent of the local lesions; neither was there a drop in the temperature of either animal. (See chart.)

Conclusion. Sulfanilamide, when given orally to rabbits in adequate doses, early in the course of experimental dermal pneumococcal infection, eliminates this microörganism from the blood stream, reduces the fever, cures the local lesion and favors recovery in most of the treated animals.

9488

Presence in Milk of the Extrinsic Factor of Castle.

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The extrinsic factor of Castle has been found in several protein-containing foods, such as beef muscle,[1] autolyzed yeast,[2] wheat germ,[2] and egg white.[3] From Castle's[4] more recent work, one may infer that some of the negative results obtained with protein foods other than these, were due to prolonged incubation periods, or the trial use of the materials at hydrogen ion concentrations too great for the interaction of the extrinsic and intrinsic factors. Casein[5] has been reported as giving a negative response at a pH of 2.5 to 3.5, however, more recently Taylor, *et al.*,[6] have indicated that the intrinsic factor is an enzyme which has optimum action at pH 7.4 to 7.7. Because infants may be fed on whole milk alone for long periods of time without developing pernicious anemia, it would seem possible that milk which contains Caseinogen as the greatest part of its protein might contain the extrinsic factor. Because of this we have investigated the value of milk as a source of extrinsic factor with 2 pernicious anemia patients, and to 3 patients with pernicious anemia we have given partitions of milk with gastric juice to see if the extrinsic factor were contained in the whey.

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[1] Castle, W. B., Townsend, W. C., Heath, C. W., *Am. J. M. Sc.*, 1929, **180**, 305.

[2] Strauss, M. B., and Castle, W. B., *New Eng. J. Med.*, 1932, **207**, 55.

[3] Miller, D. K., and Rhoades, C. P., *New Eng. J. Med.*, 1934, **211**, 921.

[4] Castle, W. B., and Ham, T. H., *J. Am. Med. Assn.*, 1936, **107**, 1456.

[5] Castle, W. B., and Townsend, W. C., *Am. J. M. Sc.*, 1929, **178**, 764.

[6] Taylor, F. H. L., Castle, W. B., Heinle, R. W., and Adams, M. A., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 566.

All of these patients presented classical signs of pernicious anemia, 2 had had previous relapses and although 2 were negroes, all, subsequent to the experiments, have gone into remissions on liver therapy. The diets used were high in carbohydrates and lacking in meat, eggs, and whole cereals, while the patients were receiving experimental material. In Case 1, however, the patient prior to the experiment received a diet rich in meat to rule out dietary deficiency. The diets of those on whey and gastric juice were also restricted in the amount of milk foods.

Case 1. A colored female 41 years of age. For 7 consecutive days, each day, this patient was given 150 cc. of gastric juice and 960 cc. of whole milk. The protein in this mixture was not over 40 gm. The typical hematopoietic response occurred and may be seen in Table I.

The second patient to receive whole milk and gastric juice was Case No. 4. This was a male 53 years of age. The first day of the experiment he received 350 cc. of gastric juice with 960 cc. of whole milk; following this he received 7 daily doses of 150 cc. of gastric juice and 960 cc. of whole milk. In this case the milk and gastric juice were not incubated and at all times were kept cold. The day after the last dose of the milk and gastric juice mixture, the diet was changed to house ration but no antianemic substance was given. A typical hematopoietic response was obtained prior to treatment with liver extract.

These two patients not only showed the typical response of blood formation but also showed improvement in appetite and in general well-being.

After obtaining a reticulocyte response and an increase in RBC and Hb in Case 1, this patient was placed on a mixture of fresh whey and gastric juice. The fresh whey was made by the addition of 50 cc. of 5% HCl to 960 cc. of whole milk at 37°C. The clotted casein was removed. About 600 cc. of whey was collected and to this 150 cc. of gastric juice was added. This, then, was brought to

TABLE I.

Case No. 1.

Diet: High carbohydrate with no meat, eggs, or whole cereals.

	RBC mil.	Hb. %	Retic. %
2-8	1.32	35	.6
2-9	1.23	35	.7
2-10	1.5	38	.7

Start of 150 cc. gastric juice and 960 cc. of whole milk mixed and incubated 2 hours.

	RBC mil.	Hb. %	Retic. %
2-11	1.45	37	0.6
2-12	1.65	38	0.4
2-13	1.26	35	1.0
2-14			0.4
2-15	1.5	36	2.5
2-16	1.62	41	7.1
2-17	1.61	40	12.0

2-17 Last day of gastric juice and milk.

	RBC mil.	Hb. %	Retic. %
2-18	1.49	40	7.9
2-19	2.04	46	11.4
2-20	1.91	48	11.2
2-22	2.17	53	6.9
2-25	1.9	53	2.7

2-26 600 cc. fresh whey and gastric juice 150 cc. started.

	RBC mil.	Hb. %	Retic. %
2-26	1.89	53	2.5
3-9	2.03	61	1.2

This is the last day of whey and gastric juice. Patient then treated with liver extract.

Case No. 3.

Diet: High carbohydrate with no meat, eggs, or whole cereals.

	RBC mil.	Hb. %	Retic. %
4-27	1.35	48	.3

800 cc. whey made by adding 150 cc. gastric juice to 960 cc. whole milk; removing clot of casein.

4-28 600 cc. fresh whey and 150 cc. gastric juice

	RBC mil.	Hb. %	Retic. %
4-28	1.62	48	.5
4-29	1.60	49	.7

Same as 4-27.

	RBC mil.	Hb. %	Retic. %
4-30	1.34	43	1.1
5-1	1.41	46	0.7
5-3	1.77	52	0.5
5-4	1.57	48	2.6
5-5	1.70	52	3.3
5-6	1.69	49	4.2

Last material given this day.

5-7 House diet without anti-anemic substance.

	RBC mil.	Hb. %	Retic. %
5-7	1.66	53	2.9
5-8	1.66	53	2.3
5-10	1.70	53	1.4

Case No. 2.

Diet: High carbohydrate with no meat, eggs, or whole cereals.

	RBC mil.	Hb. %	Retic. %
4-9	1.89	47	
4-10	1.34	49	.3
4-12	1.61	47	.4

600 cc. of fresh whey from 960 cc. of whole milk and 150 cc. gastric juice daily. 4-11.

	RBC mil.	Hb. %	Retic. %
4-13	1.89	50	.3
4-14	2.01	51	.3
4-15	1.99	51	.2
4-16	2.11	53	.8
4-17	1.96	57	.8
4-18	2.02	56	.8

60 gm. of dried whey substituted for fresh whey daily.

	RBC mil.	Hb. %	Retic. %
4-19	2.05	57	0.7
4-20	2.12	58	1.8
4-21	2.17	60	1.3

Dried whey increased to 100 gm. daily.

	RBC mil.	Hb. %	Retic. %
4-22	1.89	52	1.5
4-23	2.09	59	1.0
4-24	1.87	51	0.9

Dried whey and gastric juice observed to be negative and discontinued. 4-25.

Case No. 4

Diet: High carbohydrate with no meat, eggs, or whole cereal.

	RBC mil.	Hb. %	Retic. %
7-16	1.27	41	1.3
7-17	1.4	38	0.8

7-18 350 cc. gastric juice and 960 cc. whole milk not incubated but kept cold.

After this day, 150 cc. gastric juice and 960 cc. whole milk were used.

	RBC mil.	Hb. %	Retic. %
7-19	1.47	39	1.0
7-20	1.40	39	1.1
7-21	1.39	39	1.1
7-22	1.40	40	3.0
7-23	1.42	40	2.2
7-24	1.45	40	3.0
7-25			4.0

Last day of gastric juice and whole milk.

	RBC mil.	Hb. %	Retic. %
7-26	1.43	41	2.2

Started on house diet without anti-anemic substance.

	RBC mil.	Hb. %	Retic. %
7-27	1.68	41	5.0
7-28	1.77	50	7.9
7-29	1.72	51	10.3
7-30	1.69	47	9.4

7-30 Started on liver therapy.

a pH of 6.8-7.0 by the addition of 10% NaOH, usually about 10-15 cc. This mixture was kept cold and 750 cc. of it was fed the patient daily for 12 days. On this mixture there was no further increase in red count nor subsequent rise in reticulocytes.

This latter experiment was repeated with Case No. 2, a colored male aged 27 years. At first he was given the fresh whey and gastric juice mixture outlined above, but after 6 days 60 gm. of dried whey was substituted for the 600 cc. of fresh whey and this was increased after 3 days to 100 gm. daily. The mixture of dried whey and gastric juice was made up to about 800 cc. in volume and the pH was adjusted to 6.8 to 7.0 by the addition of NaOH. This mixture was given for 6 days in all, which was 12 days from the start of fresh whey and gastric juice. In this time, no hematopoietic response was obtained, and the patient felt weaker, although there was no drop in blood levels. On liver extract therapy he accomplished a rapid recovery.

In these 2 cases the amount of protein given in the whey and gastric juice mixture was very small. In the fresh whey there were not over 4 gm. a day, and in the dried whey not over 12.

Case 3. The patient was a male aged 68 years. To this patient was given the filtrate from 960 cc. of whole milk precipitated with 150 cc. of gastric juice at 37°C. He received this material for 8 days, although, owing to an error after the first dose, he received fresh whey and gastric juice for 2 days, and then the above filtrate was resumed for 7 days. At the end of the ninth day a reticulocyte rise of 4.2% was elicited but no rise in Hb or RBC and the patient felt no better and showed no signs of improvement, and the experiment was discontinued. We believe it to be negative.

In this work we have treated 2 patients showing typical signs of pernicious anemia with whole milk and gastric juice while maintaining them on a diet otherwise greatly devoid of Castle's extrinsic factor. Both of these patients exhibited fair hematological responses as evidence that the extrinsic factor was present in the milk. By contrast 3 patients did not give response on gastric juice and whey, which leads us to the conclusion that the extrinsic factor was in the casein part of the milk. However, it leaves us with a query as to what might have happened if we had given 400 gm. of dried whey, which would have equalled the protein content of the 960 cc. of whole milk.

9489 P

Mechanism of Anomaly Induction in Frog Eggs by Means of the Centrifuge.

A. M. Schechtman. (Introduced by B. M. Allen.)

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The mechanism of the production of anomalies by the centrifuge has taken on new significance since the work of Spemann and his school on the organizer and related phenomena. Apparently few investigators have studied centrifuged eggs in the light of this work. The eggs of *Hyla regilla,* the tree frog, have proven especially sensitive to centrifugal force, yielding highly specific accessory structures (tails and ectodermal proctodea) in as high as 100% of the specimens, if treated at the correct stage of development. Electric and hand centrifuges were used. High percentages of accessory tails and ectod. ani appear after application of centrifugal forces corresponding to 1300-2000 revolutions per minute, continued for periods ranging from 5 to 120 seconds (centrifuge arm, 11.5 cm.). Susceptibility to these forces of the centrifuge is very limited. Secondary tails and ani are not observed until the eggs treated are *advanced blastulæ* (about 3 hours before the onset of gastrulation). From this time on the percentage of anomalous embryos increases, reaching a maximum (90-100%) at about the time when the blastopore first becomes visible. Susceptibility then drops rather abruptly and is *nil* at about 2 hours after the onset of gastrulation (at temperatures 25°-28°C.).

Secondary tails present a great variety of structures: some are quite complete with chorda, somites, nerve tube, mesenchyme, sec-

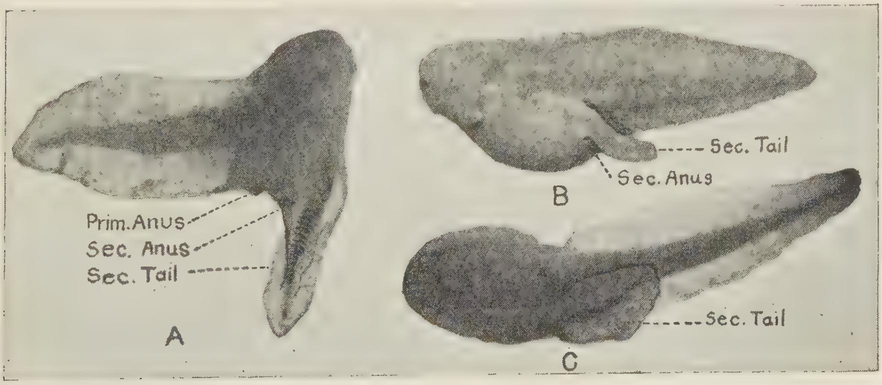

Fig. 1.

ondary anus (Fig. A); others contain only a solid axial strand (Fig. C); others consist of a flat fin with axial strand at one edge (Fig. B). Secondary ectod. ani are common (Figs. A, B).

Clear secondary tails and ectod. ani were induced by other means: (1) by pressing the presumptive ectoderm of susceptible eggs against the yolk mass, (2) by implantation of ventral or lateral blastoporal lips of the early gastrula into the blastocoele.

It seems clear from the above that the mechanism concerned in the production of the anomalies depends upon the *position* of the tail and the anal mesoderm in the blastula and gastrula. As shown by Vogt[1] the tail mesoderm is located uppermost of all the mesodermal anlagen. Collapse of the pres. ectoderm, whether by centrifuge or by mechanical pressure results in contact of the ectoderm with the organizing mesoderm. Possibly induction occurs by this contact, or portions of the mesoderm may remain adherent to the ectoderm. That the anomalies depend primarily on effects produced on the organizing centers has been suggested by others.[2, 3]

The theory of dislocation of determined ectod. anlage[4] runs counter to all recent work on the amphibia and cannot be maintained, as pointed out by Pasquini and Reverberi.[2] It seems unnecessary to postulate mal-distribution of the organizer resulting from inhibition of normal gastrulation movements,[2] since the yolk plug is engulfed in most specimens and the main axial organs are normal except for minute defects. Such inhibition, physico-chemical disturbances within the cells,[2] displacement of mitotic figures, cell injury, death,[3] or changes of cell potency, may be concerned in the production of some anomalies, especially those involving *defects,* or those arising from injury to the yolk. It is not necessary to postulate any of these factors to explain the *accessory* defects herein described.

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[1] Vogt, W., *Arch. f. Entwicklungsmech.*, 1929, **120**, 384.

[2] Pasquini, P., and Reverberi, G., *Boll. Instit. di. Zool. Univ. Roma,* 1929, **7**, 1.

[3] Beams, H. W., King, R. L., and Risley, P. L., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 181.

[4] Bagini, M., *Arch. Ital. Anat. e Embr.*, 1925, 22.

9490

Effect of Histidine on Gastric Secretion.

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The therapeutic effect claimed for histidine in the treatment of peptic ulcer seems to be based on the allegation that it prevents experimental ulcer in dogs. The exact mode of action has not been well explained. Those originally advocating the treatment claim that it probably supplies the missing product of protein digestion. However, there is no tangible evidence to show that peptic ulcer is a result of amino-acid deficiency. Martin[1] postulates the interesting theory that histidine may stimulate secretion of mucus which exerts a buffer action on the gastric juice and protects the ulcer. Volini and McLaughlin[2] actually observed in a series of 21 ulcer patients treated with this drug a uniform decrease in the amount and acid of the fasting and stimulated gastric juice. Sandweiss[3] failed to obtain similar results and could not correlate the clinical response to any change in gastric acidity. This report deals with a study of the fasting, basal, and histamine-stimulated gastric secretions following a single injection of 0.2 gm. of histidine monohydrochloride (Larostidin) in 2 normal subjects, one case of nutritional edema, 2 cases each of gastric neurosis, chronic appendicitis and chronic dysentery, and 5 cases of peptic ulcer, with various degrees of gastric acidity represented. The control and histidine observations were made on separate days but under exactly identical conditions. Histidine was injected intragluteally 30 minutes before the collection of basal secretion was begun. After 4 ten-minute samples of basal juice were obtained, histamine (Ergamine acid phosphate 0.5 mg.) was given subcutaneously and the specimen collected during the second 10-minute period was chosen as representing the stimulated secretion because the maximum acidity is usually attained in this sample. Aspiration was made continuously with a Luer syringe in order to minimize the loss of secretion through the pylorus. The volume was measured with the accuracy of one cc. For comparison of acidity only total acid was used because the free HCl titer varied considerably with the amount of blood present and with the continuous aspiration technic it was extremely difficult to avoid trauma.

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[1] Martin, K. A., *J. A. M. A.*, 1936, **106**, 1468.

[2] Volini, I. F., and McLaughlin, R. F., *Med. Rec.*, 1935, **141**, 364.

[3] Sandweiss, D. J., *J. A. M. A.*, 1936, **106**, 1452.

TABLE I.

Volume and acidity of gastric secretion with and without histidine injection. Basal juice represents the average ten-minute sample and post-histamine juice always the second ten-minute specimen. All samples obtained by continuous aspiration.

	Control observation						After injection of histidine												
	Fasting juice		Basal juice		Post-histamine juice		Fasting juice				Basal juice				Post-histamine juice				
							Volume		Acidity		Volume		Acidity		Volume		Acidity		
Case No.	Vol. cc.	Acidity N/10%	Vol. cc.	Acidity N/10%	Vol. cc.	Acidity N/10%	cc.	% Diff.	N/10%	% Diff.	cc.	% Diff.	N/10%	% Diff.	cc.	% Diff.	N/10%	% Diff.	Diagnosis
1	80	59	25	103	35	107	65	—18.8	71	+20.3	24	—4.0	90	—12.6	45	+28.6	112	+4.7	Gastric neurosis
2	44	50	9	65	18	108	45	+2.3	65	+30.0	15	+66.7	41	—36.9	26	+44.4	71	—34.2	Chronic dysentery
3	72	73	32	84	40	106	49	—31.9	75	+2.7	16	—50.0	87	+3.6	38	—5.0	94	—11.3	Duodenal ulcer
4	205	70	39	78	46	92	220	+7.3	51	—27.2	40	+2.5	69	—11.5	46	0	74	—19.6	Gastric ulcer
5	21	51	7	77	18	101	13	—38.0	20	—60.8	4	—42.8	74	—3.9	14	—22.2	109	+7.9	Gastric neurosis
6	171	57	47	76	62	90	158	—7.6	62	+8.8	53	+12.8	76	0	48	—22.6	90	0	Gastric ulcer
7	50	39	27	84	33	97	31	—38.0	47	+20.5	18	—33.3	76	—9.5	33	0	93	—4.1	Normal
8	40	18	8	20	34	62	24	—40.0	6	—66.6	6	—25.0	6	—70.0	13	—61.8	64	+3.2	Chronic dysentery
9	105	52	17	56	32	80	95	—9.5	48	—7.7	10	—41.2	49	—12.5	38	+18.8	76	—5.0	Normal
10	120	56	10	52	25	98	85	—29.2	36	—35.7	11	+10.0	53	+1.9	27	+8.0	96	—2.0	Chronic appendicitis
11		72		84		110			43	—40.2			56	—33.4			110	0	Duodenal ulcer
12	43	37	20	48	21	78	57	+32.6	50	+35.1	22	+10.0	74	+54.2	13	+38.1	84	+7.7	Chronic appendicitis
13	27	2	13	25	10	41	27	0	4		11	—15.4	22	—12.0	10	0	34	—17.1	Nutritional edema
14	105	72	20	91	32	100	*86	—18.1	78	+8.3	34	+70.0	78	—14.3	41	+28.1	84	—16.0	Duodenal ulcer
							*174	+65.7	99	+37.5	18	—10.0	92	+1.1	20	—37.5	105	+5.0	

*These examinations were made on the 3rd and 6th days of histidine treatment (0.2 gm. of histidine monohydrochloride intramuscularly, once a day) respectively.

Table I summarizes the results. No consistent change in the volume or acidity of the different phases of the gastric secretion is noted as a result of histidine injection. It might be argued that these observations were made after a single dose of histidine and therefore might not detect the effect of the drug on repeated administration. The patient in Case 14 received this medicine on 6 consecutive days and the study carried out on the 3rd and 6th days, respectively, also yielded negative results. Incidentally, this patient derived no apparent benefit from the therapy, while a standard Sippy regimen which soon followed brought on the usual expected remission.

It may thus be concluded that under the conditions of these observations histidine does not seem to exert any influence on the gastric acidity or the rate of gastric secretion.

9491 P

Contraction of Muscle and Denaturation of Myosin.

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It is recognized that the protein myosin takes part directly in the contraction of muscle, and there is even reason to believe that myosin itself is the contractile substance of muscle.[1, 2] If so significant a rôle in contraction is ascribed to myosin, it is important to know how myosin changes during contraction. Experiments concerning the effect of heat on both muscle and myosin show that, under some conditions at least, shortening of muscle may be made possible by the denaturation of myosin. In this note I shall briefly describe these experiments.

A thermal stimulus can cause either a reversible or an irreversible shortening of muscle. If the sartorius muscle of a frog is gradually warmed, its length remains unchanged until a temperature of about 37° is reached, when the muscle suddenly shortens. On cooling, the muscle recovers its original length. If the muscle is heated to higher temperatures a further shortening is observed, beginning at 39° and reaching an end-point at about 45°C. This time the muscle does not relax on cooling. The 2 responses of muscle to heat were first

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[1] Weber, H. H., *Ergeb. d. Physiol.*, 1934, **36**, 109.

[2] Muralt, A. J., *Ergeb. d. Physiol.*, 1935, **37**, 406.

sharply distinguished from each other by Jensen,[3] who referred to the reversible shortening as *thermal contraction* and to the irreversible shortening as *thermal rigor.*

In experiments on the effect of heat on myosin isolated from frog muscle I find that heat denatures myosin in 2 distinct steps, one of which can be identified with thermal contraction and the other with thermal rigor. In these experiments myosin was in a medium bearing some resemblance to its environment in the muscle fiber—in isotonic KCl with a small amount of phosphate adjusting the pH to 6.9. Under these conditions very little protein is in solution and myosin has the appearance of a transparent semi-fluid gel. When such a preparation is heated to 37° no very striking change in appearance is observed, although on more careful comparison with an unheated sample it is seen that the gel is darker and less transparent. On examining the solubility of myosin heated at 37° it is found that a pronounced change has indeed taken place. If unheated myosin is added to an excess of cold 1.2 M KCl brought to pH 8.0 with K_2HPO_4 practically all the protein dissolves, but much remains insoluble if the myosin is heated to 37° and the quantity of insoluble protein is subsequently estimated by adding 1.2 M KCl in the cold. The temperature coefficient of this change is of the order of a thousand reckoned for a change of 10°, so that at 39° a few minutes' heating suffices to render nearly all the myosin insoluble. Under these conditions there is only a slight change in the SH groups of myosin. As myosin is heated to temperatures over 39° a striking change appears. Beginning at about 41° (the exact point depending upon the rate of heating) the gel draws together into a number of firm opaque clumps. Changes in the SH groups of myosin can now be detected, and by estimation[4] of SH groups the temperature coefficient of this process has been found to be much lower than that of the first process. Both the change in solubility observed at 37° and the change in SH groups observed at higher temperatures may be regarded as steps in the denaturation of myosin because in other proteins, notably egg albumin, it has been found that loss of solubility, appearance of SH groups and an extraordinarily high temperature coefficient are the most striking characteristics of the process of heat denaturation. In egg albumin loss of solubility and change in SH groups appear to occur at the same time.[5] I shall refer to the 2 steps in the denaturation of myosin as primary (at 37-38°) and secondary denaturation (40-45°).

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[3] Jensen, P., *Arch. ges. Physiol.*, 1914, **160**, 333.

[4] Mirsky, A. E., and Anson, M. L., *J. Gen. Physiol.*, 1935, **18**, 307.

[5] Mirsky, A. E., and Anson, M. L., *J. Gen. Physiol.*, 1936, **19**, 427.

Primary denaturation of myosin can be identified with thermal contraction and secondary denaturation with thermal rigor. At the temperature of thermal contraction it is found that myosin in muscle becomes insoluble with only slight changes in its SH groups, and in thermal rigor the protein SH groups of muscle change as they do in secondary denaturation. With respect to opacity, hydration and other qualities, significant comparisons can be made between contraction of muscle and the denaturation of myosin. Experiments on rabbit muscle are of interest because in this case heat produces changes similar to those observed in the frog but at different temperatures. In the skeletal muscles of the rabbit the first effect of heat on contraction was stated by Claude Bernard[6] to appear at 45° (in contrast to 37° for the frog). I find that the primary denaturation of isolated rabbit myosin takes place at about 45°. The significance of the difference between the myosins of frog and rabbit is emphasized by comparing the myogens of frog and rabbit muscle. Myogen is an albumin-like protein in muscle, no change in which is detectable as a consequence of contraction. The myogens of frog and rabbit are denatured at the same temperature, 54°, and in this protein loss of solubility and change in SH groups occur in one step.

In contraction of muscle due to other stimuli than heat there are reasons to suppose that primary denaturation of myosin occurs; the extent of shortening in thermal contraction is of the same order as that produced by electrical stimulation;[3] and in those types of contraction in which the changes in myosin have been investigated there is observed a loss of solubility without change in SH groups.[7]

Summary. Myosin isolated from frog muscle is denatured by heat in two distinct steps: the first, occurring at 37°, is characterized by a high temperature coefficient (about a thousand for 10°) and no change in SH groups; the second, occurring between 40°-45°, has a much lower temperature coefficient and a marked change in SH groups. In the thermal stimulation of muscle two different kinds of contraction occur, a reversible contraction at 37° and an irreversible contraction at higher temperatures. The reversible contraction can be correlated with the first step in denaturation of myosin, and the irreversible contraction can be correlated with the second step in denaturation of myosin.

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[6] Bernard, Claude, referred to on page 194 in Kühne, W., *Myologische Untersuchungen*, Leipzig, 1860.

[7] Mirsky, A. E., *J. Gen. Physiol.*, 1936, **19**, 559.

9492

Effect of Ultraviolet Irradiation on Poliomyelitis Virus in Vitro.*

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The experimenter who is confronted with the problem of natural resistance to poliomyelitis finds two avenues open for methodical approach. He can either investigate the agencies which are liable to cause changes in virulence of the etiological agent or he can attempt to study and correlate the different factors which modify susceptibility of the host. While there are reasons to suspect that host factors are more important in determining the course of events, the environmental conditions which may affect the virulence of the virus obviously lend themselves more readily to experimental analysis. It is a simple matter, for instance, to expose the virus to various physical and chemical agencies and to test for its survival or destruction by intracerebral injection into monkeys. Such studies have already contributed valuable data that are helpful in explaining the nature of the infectious agent. Thus, it has been shown that poliomyelitis virus possesses a very low thermal deathpoint[1] and that it is extraordinarily susceptible to the oxidizing or reducing action of certain chemicals.[2] On the other hand, the virus has proved quite resistant to exposure, to freezing,[3] drying,[3] X-rays,[4] as well as sonic vibrations,[5] and remains relatively unharmed after contact with strong protoplasmic poisons.[6] Judging from these properties, the virus of poliomyelitis seems to resemble much more the toxins and ferments than the common animate agents of disease.

The literature offers very little information concerning the effect

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* Aided by grants from the Rockefeller Foundation and the President's Birthday Ball Commission for Infantile Paralysis Research.

[1] Shaughnessy, H. J., Harmon, P. H., and Gordon, F. B., *J. Prev. Med.*, 1930, **4**, 149.

[2] (a) Jungeblut, C. W., *J. Exp. Med.*, 1935, **62**, 517; (b) *Poliomyelitis Monograph,* International Committee for the Study of Infantile Paralysis, Baltimore, Williams and Wilkins Co., 1932.

[3] See 2b.

[4] Lenz, M., and Jungeblut, C. W., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 814.

[5] Scherp, H. W., and Chambers, L. A., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 495.

[6] Flexner, S., Clark, P. F., and Amoss, H. L., *J. Exp. Med.*, 1914, **19**, 205. See also 2b.

of ultraviolet light on the virus of poliomyelitis *in vitro,* although the light sensitiveness of other filtrable viruses, toxins and ferments has been well established. According to Flexner,[7] poliomyelitis virus is rapidly killed by sunlight, and Amoss[8] states that light in the presence of eosin destroys the virus. Schultz[9] found that exposure to ultraviolet rays from a cold quartz lamp inactivates the virus in less than 3 minutes. It was, therefore, desirable to add to our knowledge on the properties of the virus by investigating in detailed experiments the influence of ultraviolet irradiation on poliomyelitis virus *in vitro.*

The experimental setup was as follows: Amounts of 2 cc. of a 1:20 dilution of a 10% centrifuged virus suspension (Aycock strain) were placed in rock crystal vessels which were covered with a lid made of the same material. This amount of virus was chosen as representing a dose which, while not excessively large, has proved to be sufficiently infective to bring down 95% of the controls with paralysis in routine intracerebral tests. These vessels were then exposed to ultraviolet irradiation for various lengths of time (5 seconds to 30 minutes), at a distance of about 25 cm. from the quartz burner. An ultraviolet lamp, energized by high frequency currents (Lepel) and emitting a spectrum in which the longer ultraviolet waves in the range from 2800 to 3100 Angstrom units predominate, was used for this purpose. The temperature was carefully checked

TABLE I.
Effect of Ultraviolet Irradiation on Poliomyelitis Virus *in vitro.*

Exposure of virus	Test Monkey intracerebral injection (1 cc. 1:20 dil. 10% virus) Rayed	Result	Control Monkey intracerebral injection (1 cc. 1:20 dil. 10% virus) Unrayed	Paralysis days
30 min.	W95	No paralysis	W93	6
” ”	Y98	” ”	Y73	9
15 ”	X27	” ”	X23	8
” ”	Y99	” ”	Y73	9
5 ”	X61	” ”	X60	11
” ”	Y100	” ”	Y74	9
2 ”	Y5	” ”	Y6	12
” ”	Z1	” ”	Y75	13
1 ”	Z2	” ”	Y76	11
” ”	Z3	” ”	Y77	7
5 sec.	Z4	Paresis, 15 days	Y76	11
” ”	Z5	Paralysis, 14 days	Y77	7

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[7] Flexner, S., Rockefeller Institute pamphlet, 1916.

[8] Rivers, T. M., *et al., Filtrable Viruses,* Baltimore, Williams and Wilkins Co., 1928.

[9] Schultz, E. W., *J. Ped.,* 1932, **1**, 358.

in the rayed fluid during the experiment and found not to exceed 38.5°C. after one hour exposure. It is important to mention this point since poliomyelitis virus is attenuated or killed by heating for 30 minutes between 42.5 and 50°C. After raying, one cc. of the virus suspension was injected intracerebrally into monkeys. Duplicate tests were done at all intervals and each experiment was accompanied by control animals injected with one cc. of the unrayed virus suspension. The results obtained are given in Table I.

It may be seen from Table I that raying for periods of time varying from 30 minutes to as little as one minute was evidently sufficient to destroy the virus completely. Even an exposure as short as 5 seconds had possibly caused a certain degree of attenuation of the virus as suggested by the slight paresis developing in one of the 2 monkeys which had received this material.

It follows from these experiments that poliomyelitis virus *in vitro* is incredibly sensitive to the effect of ultraviolet irradiation. The mechanism of this inactivation is unknown, but it is more than likely that oxidation plays an important part. In applying this observation to the epidemiology of the human disease caution is necessary since one is dealing with a complex problem involving operation of the same factor on the virus as well as the host—and the effects produced may not be the same. It is conceivable that exposure to ultraviolet light, under certain conditions, may tend to increase the susceptibility of the host while at the same time causing a diminution in the virulence of the virus.

After completion of this work, a paper by Toomey[10] appeared, who found in repeated tests that exposure for 75 minutes at 18 inches distance from a standard quartz lamp bulb inactivated a 1% suspension of poliomyelitis virus. No shorter periods of exposure were tested.

9493

An Apparatus for Grinding Bacteria at Low Temperatures.

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The disrupting of bacteria by grinding in mills is a well established laboratory procedure, for which various forms of apparatus have been devised. The long grinding required to break most of the bacteria introduced into an ordinary mill changes the material so that some of the immunologically detectable components are no longer present, or at least not identifiable. In order to take advantage of the brittleness of cells at low temperature, Macfadyen and Rowland[1] used a grinding apparatus that was immersed in liquid air. Johlin and Avery[2] constructed a heavy steel cylinder in which a moist bacterial suspension was frozen with CO_2 ice; a tightly fitting plunger was introduced and struck 25 times with an 8 lb. hammer. These authors presented data indicating that this was a very efficient method of rupturing bacteria and obtaining the intracellular contents. For extracting tissues, a somewhat similar apparatus devised by Graeser, Ginsberg, and Friedemann[3] was used by Fox[4] to study the concentration of antibodies in tissues.

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[10] Toomey, J. A., *Am. J. Dis. Child.*, 1937, **53**, 1490.

Recently Mudd, Shaw, Czarnetsky and Flosdorf[5] have devised an apparatus for disrupting bacteria at low temperatures after desiccation by the lyophil process. The grinding chamber consists of a hollow monel metal disc, milled out in such a manner that 2 large stainless steel balls 1½ inches in diameter fit accurately into the hollow. The bacteria are placed in this mill, frozen and dried by attaching it, with airtight connections, to a condenser which is immersed in a low-temperature freezing bath. Following the drying, the mill is rotated in a similar bath of CO_2 ice and methyl cellosolve. At least 80% of the bacteria are completely disintegrated within a short time. These authors[5] report that the combination of low-temperature desiccation and grinding is specially efficacious in preventing denaturation of the bacterial components. Another feature of the apparatus is that it probably prevents scattering of dried, and living, bacteria during the processes described.*

A few years ago Kreuger[6] devised a mill consisting of a tin-lined steel cylinder of 1-liter capacity, in which were placed 2 or 3 thousand ¼-inch rustless steel balls. Bacteria in suspension, rotated in this mill, at a rate of 130 R.P.M., at room temperature, were disintegrated in 12 to 18 hours so that 30 to 40% of the intracellular nitrogen was made soluble. No detailed data were presented concerning the antigenic state of these soluble substances.

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[1] Macfadyen, A., and Rowland, S., *Centr. Bakt. Orig.*, 1903, **34**, 765.

[2] Johlin, J. M., and Avery, R. C., *J. Exp. Med.*, 1930, **52**, 417.

[3] Graeser, J. B., Ginsberg, J. E., and Friedemann, T. E., *J. Biol. Chem.*, 1934, **104**, 149.

[4] Fox, J. P., *J. Immunol.*, 1936, **31**, 293.

[5] (a) Mudd, S., Shaw, C. E., Czarnetsky, E. J., and Flosdorf, E. W., *J. Immunol.*, 1937, **32**, 483; (b) Mudd, S., Czarnetsky, E. J., Pettit, H., and Lackman, D., *Proc. Am. Philosophical Soc.*, 1937, **77**, 463.

* We are indebted to Dr. Stuart Mudd for sending us the plans and specifications for this apparatus.

[6] Kreuger, A. P., *J. Infect. Dis.*, 1933, **53**, 185.

For many years we have used a mill designed by White[7] for grinding bacteria which had been previously desiccated after freezing. This mill consists of a heavy glass flask of 1-liter capacity containing 6 or 8 agate balls; the flask rotates on an axis of 45° from horizontal. Several weeks of grinding are required for complete disruption of hemolytic or non-hemolytic streptococci which have been previously frozen and dried, and still longer for those that had been simply dried. As already noted, the material obtained after this long grinding had lost many of the antigenic properties of the original bacteria.

After Mudd and his co-workers reported their success in obtaining type-specific substances of hemolytic streptococci by disrupting of the "lyophil" bacteria in a cold mill, we thought that the advantages of the various mills might be combined in a form which could be easily and inexpensively assembled.

The flask-holder, C, of the White mill† is mounted on one end of a countershaft, about a foot long, which runs horizontally in 2 bearings (not shown), one of which is situated about 3 inches from the holder; and on the middle of the shaft is placed a grooved pulley of such size that it will reduce the speed from an electric motor to about 230 R.P.M. The grinding unit, which may be sterilized with dry heat, consists of a heavy short-necked Pyrex flask, F, of 1-liter capacity, containing about 500 ¼-inch stainless steel balls. Less effective grinding is obtained with 1000 balls because the larger mass does not oscillate so freely. After the material to be ground is inserted, the flask is closed as follows: A gum-rubber stopper of suitable size is placed inside a piece of sterile gauze or oiled silk, and pushed into the neck of the flask until the inner end of the stopper is even with the inside surface of the latter; a second slightly larger stopper is also inserted; this usually projects a short distance from the top of the neck. The gauze is trimmed flush with the second stopper, over which is placed a gum-rubber milk-bottle cap, K, held closely to the neck of the flask with tightly stretched wide rubber bands. This prevents the methyl cellosolve from making contact with the stopper. The flask is held firmly in place by 3 windings of spring belting fastened by wire loops slipped over the end of the heavy wire arms of the flask-holder. A smaller holder is available for a flask of 200 cc. capacity.

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[7] White, B. J., *Centr. Bakt. Orig.*, 1909, **48**, 254.

† This is a stock article obtainable from Arthur H. Thomas Company, Philadelphia.

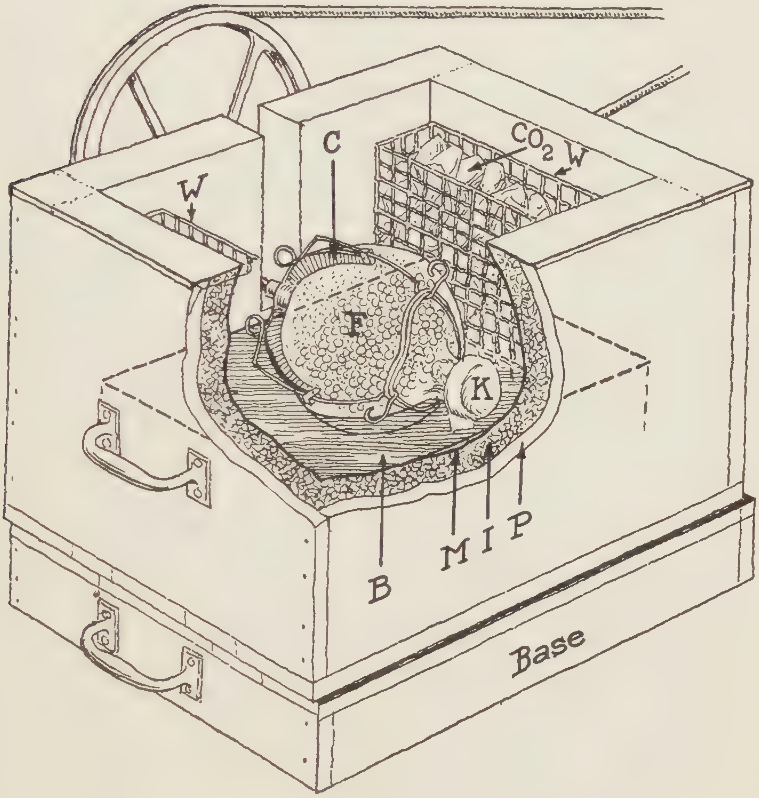

FIG. 1.

The flask is rotated in a bath, B, of methyl cellosolve cooled with CO_2 ice. A lower temperature is maintained and a smaller amount of the ice is used if the bath is properly insulated. A copper box, M, 13 inches long, 10 inches wide and 9 inches deep, with a slot 1 inch wide and 4 inches deep in the middle of one side, is a convenient size for the bath. Around this metal box is one inch of cork board, I, which, in turn, is covered with a casing of pine, P. The metal extends over the top of the cork and pine and also lines the slot, in order to prevent the methyl cellosolve from coming in contact with the cork. About the upper side of the box is nailed a strip of board, 3 inches wide, which thus forms a recess into which fits a removable metal top similarly insulated with cork and pine (not shown in the figure). In each end of the metal box is placed a removable wire basket, W, 2½ inches wide, 10 inches long and 8 inches deep. which is filled with pieces of CO_2 ice that are thus kept from contact with the rotating flask. The refrigerating box stands on a removable base; this permits convenient separate manipulation of the countershaft, flask-holder and box.

Before grinding, the bacteria are frozen and dried, either in the lyophil apparatus of Flosdorf and Mudd,[8] or by the desiccator-method.[9] Weighed amounts are then placed in a flask, which is tightly stoppered as previously described, and cooled to —75°C. in the bath used for the mill. If the flask is stoppered before chilling and opened while still cold, there will be a rush of air which will blow the bacterial powder inward from the neck of the flask, instead of into the surrounding atmosphere, an occurrence that will take place if this sequence is not followed.

After being properly chilled the flask is firmly fastened in the holder,† so that it is immersed about an inch in the methyl cellosolve. The top is placed on the box and the motor started. The large masses of dried bacteria are quickly broken up; and the bacteria form a coating over the inner surface of the flask and around the balls. The latter act like hundreds of little hammers which hit and grind the cold, brittle bacteria. It has been found that hemolytic streptococci, in a quantity of 1 gm. dry weight, are effectively disrupted after one to 3 hours' grinding in this cold mill.

This technic has been found superior to grinding bacteria which have been frozen in a thin film on the inner surface of the flask without preliminary drying, a method which is somewhat comparable to that described by Macfadyen and Rowland or by Johlin and Avery. The results of studies of material obtained from desiccated bacteria ground in this mill will be reported later.

9494 P

Photodynamic Action of Methylene Blue on Poliomyelitis Virus.*

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From the Laboratories of the Infantile Paralysis Commission of the Long Island College of Medicine, and the Jewish Hospital of Brooklyn.

Reitz[1] reviewed the effects of the combined action of light and dyes on a number of bacteria, and found that methylene blue, among other dyes, was a very active photodynamic agent. More recently,

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[8] Flosdorf, E. W., and Mudd, S., *J. Immunol.*, 1935, **29**, 389.

[9] Swift, H. F., *J. Bact.*, 1937, **33**, 411.

* This work supported by grants from the Friedsam Foundation and from the President's Birthday Ball Commission for Infantile Paralysis Research.

[1] Reitz, A., *Zentralbl. f. Bakt.*, 1908, **45**, 270, 374, 451.

Tung[2] has also studied the photodynamic effect of methylene blue on a number of microörganisms. Perdrau and Todd[4] have reported on the photodynamic effect of methylene blue on a number of viruses including vaccinia, herpes, and canine distemper. Their method consisted of exposing dilute mixtures of methylene blue and the virus filtrate to the radiation of a 100 c.p. pointolite light, at a distance of 20 cm. from the petri dishes which contained the mixtures in 2 mm. layers. The final methylene blue concentrations used varied from 1:10,000 to 1:100,000 and the exposure times ranged from 5 to 30 minutes. After irradiation, the infectivity of the mixtures was tested in animals. Their results indicated that most of the viruses studied were completely inactivated by the above treatment. Perdrau and Todd[4] furthermore observed that although the virus of canine distemper was inactivated, it remained antigenic, and animals treated with this inactivated virus did not develop signs of the disease but showed considerable resistance to infections with the active virus. Shortt and Brooks,[5] however, could not demonstrate any immunity produced by inoculating animals with photodynamically inactivated rabies virus.

This report deals with the photodynamic action of methylene blue on the virus of poliomyelitis.

The methods employed were essentially those of Perdrau and Todd.[3, 4] The methylene blue was Grubler's Methlenbalu, f. Bac. Koch, which was autoclaved when made up to twice the required dilution with saline (See Table I). To insure uniformity only small amounts were made up at frequent intervals.

The virus was obtained from spinal cords of monkeys that were sacrificed at the height of the disease. Cord fragments were ground with sand in physiological saline solution to make 5% suspensions and filtered through Seitz filters. The following manipulations were carried out in a photographic dark room by the light of an orange safelight. Equal amounts of virus suspensions and prepared dilutions of methylene blue were thoroughly mixed. Ten cc. of each of the mixtures was pipetted into sterile petri dishes, thus forming a layer of about 2 mm. at the bottom of each dish. For exposures, the dishes were placed on white paper and the lids slightly raised to prevent a film of water of condensation forming on the upper lids. However, if clouding resulted, the top lids were replaced with dry, sterile ones. Subsequent experiments showed that exposures in a

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[2] Tung, T., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 328.

[3] Perdrau, J. R., and Todd, C., *Proc. Roy. Soc. B.*, 1933, **112**, 288-298.

[4] Perdrau, J. R., and Todd, C., *J. Comp. Path. and Therap.*, 1933, **46**, 79.

[5] Shortt, H. E., and Brooks, A. G., *Indian J. Med. Res.*, 1935, **22**, 557.

TABLE I.
Photodynamic Action of Methylene Blue on Poliomyelitis Virus.

Date	Monkey No.	Methylene Blue Final Dilution	Time Exposed	Outcome
		Experiment I.		
1/5/37	J249	1:50,000	30 min.	Survived
	J250	”	30 ”	Prostrated, sacrificed in 12 days
	Controls			
	J251	”	Not exposed	” ” ” 11 ”
	J252	Saline	30 min.	” ” ” 7 ”
	J253	”	Not exposed	” ” ” 6 ”
		Experiment II.		
2/5/37	J259	1:50,000	45 min.	Survived
	J260	”	45 ”	Prostrated, sacrificed in 14 days
	Controls			
	J261	”	Not exposed	” ” ” 8 ”
	J262	Saline	” ”	” ” ” 8 ”
		Experiment III.		
4/7/37	J268	1:75,000	60 min.	Survived
	J267	”	60 ”	”
	J271	1:100,000	60 ”	”
	Control			
	J274	Saline	Not exposed	Prostrated in 10 days; sacrificed in 19 days
		Experiment IV.		
4/21/37	J301	1:75,000	45 min.	Prostrated, sacrificed in 10 days
	J303	”	60 ”	Survived
	J302	1:100,000	45 ”	Prostrated, sacrificed in 14 days
	J304	”	60 ”	Survived
	Control			
	J300	Saline	Not exposed	Prostrated, sacrificed in 6 days

dry, well ventilated room did not produce clouding. The source of light was a new 100 watt bulb placed directly above the plates at a distance of 20 cm. (As indicated in Table I, time and concentrations of dye were varied.) Following exposure, 2 cc. was removed from each plate and kept in a blackened test tube until inoculated intracerebrally into *Macacus rhesus* monkeys. Those animals that succumbed were autopsied and the diagnosis confirmed by histologic study.

The following controls were included: (a) for light, unexposed mixtures of virus and methylene blue, (b) for dye, saline was substituted for methylene blue, (c) for virulence, unexposed plates of equal parts of saline and virus. Experiments in which the virulence controls did not succumb within the incubation period were excluded.

Table I gives the results of the experiments. It will be noted that methylene blue in concentrations from 1:50,000 to 1:100,000 will inactivate the virus of poliomyelitis when exposed to the light of a 100 watt bulb under the experimental conditions. Inactivation was most constantly obtained with dilutions of 1:100,000 of the dye and an exposure of 60 minutes.

9495

Toxic Effects of an Excess of Vitamin B_1 in Rats.

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Incidental to other studies on the effect of an excess of vitamin B_1 on resistance, certain unusual toxic manifestations of an excess were observed. We have been unable to find any report in the literature describing similar effects.

The rats used in these experiments were of Wistar stock, raised in our laboratories and free from *Bartonella muris*. Three groups of animals, 4 weeks old, were used. All 3 groups were fed a complete diet consisting of 15 gm. per rat per day of a basic mixture of hominy 100 parts, rolled oats 25 parts, fine meat and bone 25 parts, salt 1½ parts and dried skimmed milk 16 parts, to which was added a few drops of cod liver oil, 0.3 mg. of wheat germ and 0.3 gm. of crude Fleischman's brewer's yeast per rat. This yeast product contains 6 I.U. of B_1 per gram. This is equivalent to about 2 I.U. per rat per day. In addition, the animals received fresh greens twice a week and fresh milk every day. On this complete diet, it has been our experience that the rats maintain a good growth curve, reproduce well and rear all their young without loss of any members of their litters.

In the present experiment, one group containing 8 females was fed this complete diet, but supplemented with Mead Johnson's brewer's yeast in amounts equivalent to 50 I.U. of vitamin B_1 per rat per day. A second group containing 6 females was given supplements of vitamin B_1 concentrate (adsorbate, Eli Lilly) in amounts equivalent to 50 I.U. per day. The vitamin B_1 adsorbate was subsequently replaced by synthetic vitamin B_1 (Betaxin) and this was given in equivalent unitage, administered subcutaneously. A third group in which there were 9 females maintained on the standard complete diet alone, was used as controls. The experiment was continued for several generations.

Of the control group, within a period of 4 months (in the winter) 5 became pregnant and had normal litters of 7 to 10 young, all of which grew to maturity. The other 4 did not conceive in this period. The offspring continued to breed at maturity as usual. In common with the experience of others, we have observed fluctuations in the rate of reproduction dependent on season, but only rarely have we observed instances in which a litter was not normally raised.

The rate of growth of the animals fed an excess of B_1 concentrate, or synthetic vitamin B_1, was no greater than that of the group of animals fed the normal complete diet. The females of these groups bred more regularly than the controls. However, in the first generation of rats fed the excess B_1 concentrate, 2 of their 7 litters were born dead, apparently due to premature parturition; 2 were born alive, but died and were eaten within 24 hours; and 3 litters of 3 to 8 young grew to maturity. Before the second mating, the B_1 concentrate was replaced by synthetic vitamin B_1 (Betaxin) in equivalent unitage injected subcutaneously. One mother that had eaten its first litter, when given Betaxin instead of B_1 concentrate, became pregnant a second time and threw a litter of 4 healthy young that grew to maturity. The second generation on Betaxin, however, when bred, showed the same toxic effects. In 3 litters the young were born alive but the mothers neglected their young and within 48 hours had devoured most of the offspring.

The animals fed an excess of vitamin B, in the form of Mead Johnson's brewer's yeast, thrived much better than the controls or those fed vitamin B_1 concentrate as far as the rate of growth was concerned. Throughout the experiment, the animals weighed on an average of 50% more than did the members of the other 2 groups. (This is consistent with the findings of Williams, Waterman and Keresztesy[1] who noted improved size of rats fed increasing amounts of vitamin B_1 up to a limit of 100 times the minimal protective dose.) Seven of 8 females conceived and gave birth to litters of 6 to 11 young at a time of the year when breeding in the control stock was at a minimum. However, in 2 litters, the young were ignored by the mothers, and they died of starvation, a defect of lactation apparently being present. The other 5 litters grew to maturity, their growth curve exceeding the controls. This second generation grew well and showed no toxic effects from the excess. However, when they became mature, some were inbred with their litter mates and of the 9 litters of this second generation, in 2 the mothers showed no interest in the young and failed to lactate adequately. As a result, the young became emaciated and starved to death. In 4, the mothers ate the young within 24 hours. When it was noted that the first 6 litters born of the mothers in this second generation were lost, the excess yeast was discontinued for a period of 2 weeks and then readministered in amounts of 20 I.U. per day to the remaining pregnant females. Of the next litter born after this change in quantity of excess yeast administration, 3 of the young survived. The remaining young of this litter were not nursed and starved to death. Litters born within a period of 4 weeks after the reduction of the excess of yeast were perfectly normal, all surviving. The mothers lactated adequately and showed the normal interest in their young. It is interesting that new-born rats of the stock given an excess of yeast for 2 generations, injected daily subcutaneously with 20 to 50 I.U. of synthetic vitamin B_1, did not show any perceptible difference in the time of weaning from those of the control normal stock fed our standard adequate diet.

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[1] Williams, R. R., Waterman, R. E., and Keresztesy, J. C., *Science*, 1935, **81**, 535.

Since the groups receiving excess yeast, concentrated B_1 and synthetic B_1 showed similar toxic effects, it is definite that the common responsible factor was B_1 and not other elements in the B_1 adsorbate or other factors in the yeast.

Leong[2] observed that the maximum storage of vitamin B_1 is attained following the administration of 30 I.U. per rat per day in a period of 4 to 6 weeks. From our own observations, it would seem that an excess of vitamin B_1 in amounts exceeding 20 or 30 I.U. a day during a period of more than one generation may interfere with lactation and the normal maternal instinct, resulting in high litter mortality and cannibalism. Nakahara, Inukai and Ugami[3, 4] have recently described an "L-factor" in liver and yeast which they state is a specific dietary factor necessary for lactation. It is not present in the concentrated vitamin B_1 adsorbate. It is possible that vitamin B_1 in great excess may inhibit the action of such a lactation factor.

Summary. Additions of Mead Johnson's brewer's yeast in amounts equivalent to 50 I.U. of vitamin B_1 per animal per day will cause a marked increase in the weight curve for several generations. This amount of yeast, however, results in a disturbance in lactation and a loss of the nursing instinct evident in the first generation, but almost universal in the second generation, with consequent starvation of the young, and a high incidence of cannibalism. The effect was more pronounced in those animals fed B_1 concentrate (adsorbate). Among these, still-births were common as well. Synthetic vitamin B_1 was somewhat less toxic in excess amounts than the vitamin B_1 adsorbate but similar interference with lactation and the nursing instinct in the second generation occurred. It is interesting that vitamin B_1 in amounts equivalent to 40 times the maintenance requirement seems to have a similar effect in interfering with the capacity of the mother to rear her young and the nursing instinct as does a relative insufficiency of this vitamin. This should in no way discourage the generous use of vitamin B_1 in the diet of human beings, as the difference between the optimal requirement and the amount which may prove toxic after several generations is considerable. The excess amount given to our rats would be equivalent to the administration of between 5,000 and 10,000 I.U. of vitamin B_1 per day for several generations (assuming that the vitamin B_1 requirement of humans is between 100 and 200 units per day).

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[2] Peng Chong Leong, *Biochem. J.*, 1937, **31**, 367.

[3] Nakahara, W., Inukai, F., and Ugami, S., Abstr. in *Ber. ü. d. g. Physiol. u. Exp. Pharm.*, 1937, **98**, 148; *Sci. Pap. Inst. Physic. Chem. Res.*, 1935, **28**, 152.

[4] Nakahara, W., Inukai, F., Kato, S., and Ugami, S., Abstr. in *Ber. ü. d. g. Physiol. u. Exp. Pharm.*, 1937, **98**, 149; *Sci. Pap. Inst. Physic. Chem. Res.*, 1936, **29**, 47.

I am indebted to Mead Johnson for the supply of brewer's yeast for these and other experiments, to Eli Lilly for vitamin B_1 concentrate, and to Winthrop Chemical Company for the generous supply of Betaxin (synthetic vitamin B_1).

9496 P

Lysis of *Vibrio comma* by Bacteriophage and by Immune Serum.*

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The very simple idea that a bacteriophage may be a minute particulate living being capable of growing and multiplying within a larger living cell and able there to produce soluble enzymes which may act upon the bacterial structure and may eventually diffuse in solution through the medium has been postulated by d'Herelle and has been supported by ever-increasing experimental evidence assembled by his disciples. Certainly this conception is in accord with what we know about larger organisms such as the coccidia, the malarial plasmodia, and the yeasts. The morphological observations upon the bacteriophage particles have been technically difficult and the results not wholly satisfactory. The study of other filterable viruses has met with similar difficulty.

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* This work was aided in part by a grant from the Josiah Macy, Jr., Foundation. The more complete report will appear in an early number of the *Journal of Infectious Diseases*, 1937, **61**, 222.

Castaneda,[1] by a special staining technic, has been able to demonstrate quite clearly the minute rod-like Mooser bodies that develop on the serous tunic of the guinea pig's testis after inoculation with Mexican typhus fever and the resulting pictures are so definite that one is inclined to accept these minute rods as microbes and as the probable microbic cause of the disease.

In the present study the Austria strain of *Vibrio comma* was subjected to lysis by immune serum in one series of tubes and in a companion series it was subjected to lysis by a bacteriophage, kindly sent to us by Lieutenant Colonel J. Morison, I.M.S., of the Pasteur Institute at Shillong, Assam, India. At intervals of 15 to 30 minutes the bacteria were thrown down by centrifuge and the sediments were stained by Castaneda's technic.

In the microscopic study of the stained preparations from the serum-series a considerable amount of debris, apparently derived in part from the serum, was found associated with the bacterial cells undergoing lysis. The bacterial cells showed variation in intensity of staining but differentially stained granules were not recognized in any of these bacterial cells. In general the lysis by serum seemed to progress without any marked alteration in the size of the bacterial cells, although occasional large cells were seen in the serum-series as also in the controls.

In the bacteriophage-series the macroscopic appearance indicated that lysis was progressing at approximately the same rate as in the serum-series. The microscopic study of the sediments, however, disclosed striking differences. At the end of 90 minutes the bacterial cells exposed to phage were in many instances distinctly swollen and this feature became progressively more exaggerated up to 3½ hours. Along with these enormously enlarged bacterial cells there also occurred very minute bacteria, much smaller than normal and in remarkable contrast to the gigantic cells alongside. Perhaps the most interesting features were found in the changed internal structure of the bacterial cells and especially the intracellular granules which stain a deep blue color with the Castaneda stain. These granules are so minute as to appear as nebulous collections in some parts of the pale red bacterial protoplasm. Sometimes they are assembled in compact clumps a micron in diameter. Their distribution is irregular.

The possibility that these granules represent the particles endowed with the property of reproduction and with the property of secreting the lytic enzymes of the bacteriophagic principle is worthy of careful consideration.

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[1] Castaneda, R., *J. Infect. Dis.*, 1930, **47**, 416.

9497 P

Effect of Thyroxine on Oxygen Consumption of the Toadfish.

R. W. Root and William Etkin. (Introduced by J. A. Dawson.)
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It has been commonly assumed that thyroid medication increases the metabolic rate of all vertebrates. For cold-blooded animals, however, the evidence is very scanty. Negative results on frogs and several other poikilotherms have been reported.[1, 2] Positive results on metamorphosing amphibia had been frequently reported but upon critical review were found unconvincing by one of the present authors who obtained negative results.[3] It was therefore thought desirable to investigate the question in a fish where the complications of metamorphosis could be avoided.

A continuous flow apparatus was set up in which oxygen determinations were made by the Winkler technique.[4] By this method repeated determinations can be made on individual animals over a period of days without the slightest disturbance of the animals. With proper precautions for constant temperature (24°C.), elimination of light, etc., it was found that 8-24 hours after introduction into the chambers the oxygen consumption of the fish fell to a fairly constant "standard" level.

The toadfish, *Opsanus tau* (Linnaeus), was used. The animals, weighing about 350 gm., were paired off, starved for at least 3 days before and throughout the course of an experiment. The experimental animals were injected with synthetic thyroxine (disodium salt "Roche", 1 mg. per ml.). Usually 2-3 mg. were injected per day for 4-5 days. The dose was varied, however, a single massive dose of 10 mg. being tried as well as daily 1 mg. doses. The total dose varied from 10 to 23.5 mg. The controls were injected with distilled water brought to pH 11 with KOH. Oxygen determinations were made for a preliminary period of 3 days and for successive similar periods 12 to 18 days after the first injections.

For a statistical evaluation of the results the O_2 consumption of the experimental animal was divided by that of the control for each reading of "standard" metabolism. The values so obtained were

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[1] Henschel, Hans, and Steuber, Maria, *Klin. Wschr.*, 1930, **2**, 1442.

[2] Drexler, E., and Issekutz, B. v. jun., *Naunyn-Schmiedeberg's Archiv.*, 1934, **177**, 435.

[3] Etkin, William, *Physiol. Zool.*, 1934, **7**, 129.

[4] Keys, A. B., *Biol. Bull.*, 1930, **59**, 187.

statistically analyzed for the preliminary run and for each of the subsequent periods.

Results. Five pairs of animals were carried through successfully. In 4 pairs no significant effect of the thyroxine could be noted. In the fifth a rise of 28% was found at the 9th to 12th day after the first injection. This animal, however, suffered a severe tail lesion at the time of introduction into the respiratory chamber. Since the standard error of the mean of each period varied from 3 to 7% of the mean, it is apparent that a change of even 25% in the standard level would be clearly brought out in the present study. The absence of such a rise in all but one case (where its significance is perhaps doubtful) leads the authors to the opinion that, in the toadfish, thyroxine, even in heavy doses, does not materially increase the oxygen consumption of the normal animal. At the end of the experiment in 3 cases 2 to 2.5 mg. of 2-4 dinitrophenol were injected into the experimental animals. This resulted in a rise of almost 100% in the oxygen consumption, showing that the animals were capable of responding to an effective agent.

9498

Low-Iodine Goiter and the Resistance of the Rat to Thyreotropic Stimulation.

John W. Remington.* (Introduced by Robert Gaunt.)

From the Department of Biology, Washington Square College, New York University, and the Department of Nutrition, Medical College of the State of South Carolina.

The rat thyroid is peculiarly resistant to stimulation by the anterior lobe thyreotropic hormone.[1] Anderson and Collip[1] found that rats showing spontaneous goiters of unknown etiology developed a more severe hyperthyroidism after thyreotropic injections than did normal animals, which suggests that the stress of hyperplasia of the thyroid in some way alters this resistance. Severely hyperplastic thyroids can consistently be produced in the rat by means of a diet with inadequate iodine intake.[2] In this work, the modified low-iodine diet of R. E. Remington[3] was used in an effort to determine whether rats bearing hyperplastic goiters of known origin would show an augmented susceptibility to thyreotropic stimulation.

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* The author is indebted to Drs. Roe E. Remington, Robert Gaunt, and H. A. Charipper for assistance with this problem.

[1] See review in Anderson, E. M., and Collip, J. B., *J. Physiol.*, 1934, **82**, 11.

[2] Levine, H., Remington, R. E., and Von Kolnitz, H., *J. Nutrition*, 1933, **6**, 325.

Three methods of pituitary administration were used: (1) implants of freshly ground anterior lobes of beef pituitaries into the thigh muscles; (2) injections of an alkaline extract of sheep anterior lobes;† (3) injections of a more purified extract, "Phyone".† Treatment was continued for one week. The potency of all 3 materials was established by the injection of similar doses into immature guinea pigs. The beef implants were the most effective of the 3.

The criteria for judging thyroid response were the fresh weight, dry matter content, iodine content, and histological picture. Techniques used were those of R. E. Remington.[3]‡

(a) Normal Animals (Table I, Series A). Two groups of normal animals receiving pituitary substance showed a slight increase in thyroid weight, of questionable significance. Dry matter and iodine content showed a significant decrease. Histological study showed a rather wide variation in degree of response, but in general there was a heightening of epithelial cells, a partial softening and disappearance of colloid, and a tendency toward hyperplasia.

(b) Goitrous Animals (Table I, Series B, C, D). In none of the groups was there a significant increase in thyroid fresh weight or decrease in dry matter and iodine content following pituitary administration. No gross external signs of the development of a thyrotoxic condition were evident, and no difference could be seen between histological pictures of the thyroids of treated and untreated animals.

Since the lack of response of goitrous animals to pituitary stimulation might in part be due to an inadequacy of available iodine, 1 γ iodine per day (a minimal protective dose[4]) was given to 2 groups of rats, with and without pituitary administration (Series E). The untreated goitrous rats of Series D served as controls. The iodine induced an average decrease of approximately 6 mg. per 100 gm. body weight, with a partial return of the dry matter and iodine content toward normal levels. There was no significant difference in degree of recovery between the 2 groups. Histological study showed no difference between thyroids of the group receiving pituitary and those of the group which did not.

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[3] Remington, R. E., *J. Nutrition,* 1937, **13**, 223.

† The sheep extract was kindly supplied by Dr. Oliver Kamm, of Parke, Davis and Company, and the Phyone by Dr. David Klein, of the Wilson Laboratories.

‡ Iodine analyses were made by Mr. Cecil L. Smith.

[4] Levine, H., Remington, R. E., and Von Kolnitz, H., *J. Nutrition,* 1933, **6**, 347.

TABLE I.

Series	No.	Treatment	Aver. final wt., gm.	Thyroid Glands: Wt. per 100 gm. body wt., mg.	Thyroid Glands: Dry Matter, %	Thyroid Glands: Iodine, dry basis, %
	27	Normal[1]	102	10.0±0.5 5.7-14.6	33.3	.200
A	14	ASE[2]	115	11.8±0.2 10.6-14.0		
	18	PI[3]	102	11.3±0.3 8.2-15.6	26.9	.178
B	15	Untreated Goitrous[4]	150	14.8±0.3 12.1-18.0	25.6	
	12	ASE[5]	159	15.3±0.4 11.7-19.1	25.1	
C	9	Untreated Goitrous[6]	87	22.1±0.9 15.1-27.7	20.2	
	9	PI[7]	88	20.9±1.0 15.6-28.0	19.4	
D	9	Untreated Goitrous[6]	115	25.4±0.7 22.0-40.0	20.6	.008
	9	PI[7]	117	26.6±0.8 21.5-32.3	20.2	.012
	9	Phyone[8]	110	23.0±0.7 17.5-26.5	21.0	.015
E	9	PI+NaI[9]	118	19.0±0.9 12.8-26.3	22.2	.056
	9	NaI[10]	115	20.0±0.9 16.5-28.6	22.8	.055

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[1] From table of normal thyroids for colony used: Remington, R. E., Remington, J. W., and Welch, S. S., *Anat. Rec.*, 1937, **67**, 367.

[2] Alkaline Sheep Extract, 0.8 cc. per day for 7 days.

[3] Beef Pituitary Implants, daily for 6 days.

[4] Placed on Diet No. 342 (Remington, R. E., *J. Nutrition*, 1937, **13**, 223) when approximately 60 gm. in weight, injections begun at end of 35 days.

[5] Alkaline Sheep Extract, 1 cc. per day for 6 days.

[6] Born of goitrous mothers, on low-iodine diet from time of conception.

[7] Beef Pituitary Implants, daily for 7 days.

[8] Phyone, 1 cc. per day for 6 days.

[9] Beef Pituitary Implants daily for 7 days, with 1γ iodine per day, in an aqueous solution of NaI, injected interperitoneally.

[10] 1γ iodine per day for 7 days, injected interperitoneally.

Summary. 1. Normal rats receiving pituitary stimulation showed a slight positive thyroid response, as determined by differences in fresh weight, dry matter content, iodine content, and histological picture. 2. Rats with hyperplastic goiters, produced by an iodine-deficient diet, were just as resistant to pituitary stimulation as were the normals. 3. Iodine administration at a level which will just protect the normal rat against thyroid hyperplasia does not affect this resistance. 4. On the basis of the criteria used, the low-iodine hyperplastic goiter seems to differ from the reported spontaneous goiter, in that it does not alter the natural resistance of the rat to thyreotropic stimulation.

9499

The Purpuric Reaction Produced in Animals by Derivatives of the Pneumococcus.

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Julianelle and Reimann[1] have described the purpura that develops in white mice following the intraäbdominal injection of pneumococcal autolysates. This reaction is most evident in those portions of the skin on which hair is either scanty or absent. They concluded that some autolytic derivative of the bacterial cells damaged the endothelium of the capillaries in these areas.

The experiments of Julianelle and Reimann have been largely repeated and extended. One of the most striking features of the reaction is that the purpura produced is seldom evenly distributed over the ears, feet, tail, snout, and genitalia. Even on the ears the distribution may be splotchy while along the tail the reaction is always most irregular, certain points showing a very intense reaction while others may be completely negative. These irregularities as well as the anatomical distribution of purpura suggested that some factor such as a mild trauma might be involved in the localization of the reaction. As a test of this hypothesis several experiments have been carried out of which 2 typical examples may be cited.

Experiment 1. An area of one sq. cm. of the abdominal skin of a mouse was carefully shaved. Immediately thereafter 0.5 cc. of an autolysate of rough pneumococci prepared by the method of Julianelle and Reimann was injected intraäbdominally at a point removed from the shaved area. At 24 hours the shaved lesion was markedly purpuric but the removal of hair from neighboring areas showed these to be free of purpura. A second injection of the autolysate was then given. Twenty-four hours thereafter the freshly shaven areas were purpuric but further removal of hair showed that the adjacent skin was completely clear. It seems reasonable to conclude that the irritation associated with the shaving determined the localization of the purpura.

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[1] Julianelle, L. A., and Reimann, H. A., *J. Exp. Med.*, 1926, **43**, 87, 97; 1927, **45**, 609.

Experiment 2. Mice were placed in individual jars lined with cotton. After a preliminary period of 24 hours during which the animals were not handled, each mouse received an intraäbdominal injection of 0.5 cc. of an autolysate of rough pneumococci. In the course of these injections great care was taken to avoid handling the ears except as noted in Table I. From these results it will be noted that, in general, the purpuric reaction occurred in those areas previously subjected to mild irritation.

TABLE I.

Mouse	Handling	Results at 12 hours; presence of purpura Left ear	 Right ear
1	Ears avoided	+	—
2	” ”	—	—
3	” ”	—	—
4	Left ear handled, right avoided	++	—
5	Right ear handled, left avoided	—	++
6	Both ears handled	++	++
7	” ” ”	++	++
8	” ” ”	++	++

A somewhat similar predilection for cutaneous areas subjected to mild irritation is manifested by the eruptions of certain exanthemata and some forms of clinical purpura. In these instances the local pressure of clothing or of applied adhesive tape will frequently determine the sites of the initial cutaneous lesions.

Previous reports[2] have described the purpura observed in the dermal pneumococcal infection of the rabbit. This reaction not infrequently develops in the course of the infectious process and is almost invariably associated with the development of bacteriemia. The extent and intensity are sometimes increased after the intravenous administration of certain substances as, for example, a bacterial extract containing a specific enzyme.[3] This purpuric reaction in the rabbit is always confined to the locally infected area and has not been observed on other parts of the body-surface. It has now been found that if one injects pneumococcal autolysate intravenously in a dermally infected rabbit the entire dermal lesion becomes purpuric within a very short time, but that purpura occurs only in this area.

In summary, the autolysis of pneumococci under certain conditions gives rise to a substance capable of producing purpuric reactions in mice and in rabbits but the presence and location of these reactions is dependent upon the condition of the capillaries in the various cutaneous areas.

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[2] Goodner, K., *J. Exp. Med.*, 1928, **48**, 1; Goodner, K., and Rhoads, C. P., *J. Exp. Med.*, 1931, **54**, 41.

[3] Goodner, K., Dubos, R., and Avery, O. T., *J. Exp. Med.*, 1932, **55**, 393.

9500 P

Differentiation of Glomerular and Tubular Function in Glomerular Nephritis.

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It was recently shown that at low plasma levels about 94% of the phenol red clearance in the normal human kidney is accomplished by tubular activity.[1] In view of this fact this clearance is a sensitive index of tubular excretion, and when observed simultaneously with the inulin clearance, affords evidence of the respective functional activities of the tubules and of the glomeruli. The present investigation concerns the study of these clearances together with the urea clearance in 21 subjects with glomerular nephritis either during a first attack, during an exacerbation, or in the chronic stage.

We have endeavored to maintain the urine flow above the augmentation limit wherever possible so that the urea clearances would be physiologically comparable. The urea clearance has ranged from 64.6 to 5.4, the inulin clearance from 131 to 6.2 cc. per minute; the urea/inulin clearance ratio has ranged from its normal value of 0.55 to a value of 0.88. In no case has the urea/inulin clearance ratio been observed to fall significantly below the normal value in any stage of the disease. In general it tends to rise, so that in advanced chronic glomerular nephritis it has a value of 0.85 or higher. Our observations do not bear out the belief that in renal disease there is increased back-diffusion of urea, or that the elevation of the blood urea is due to such back-diffusion.

A full discussion of the behavior of the phenol red clearance and its relation to the inulin clearance in various stages of disease must be deferred to a later time, but a few interesting features may be noted here. In general, in those individuals in whom renal impairment is not too far advanced, the phenol red/inulin clearance ratio tends to maintain its normal value (above 3.0), suggesting that injury of glomeruli and tubules progresses in a parallel manner, as might be expected from the anatomical structure of the nephron. But in more advanced cases this ratio tends to fall below normal, reaching such low values as 0.60, indicating almost complete loss of the capacity of the tubules to excrete the dye. (Values less than 1.0 are to be expected in theory since much of the plasma phenol red is rendered unfilterable by being bound to plasma proteins.) In no case has there been evidence of the persistence of functioning aglomerular tubules in advanced chronic glomerular nephritis, as judged by the excretion of phenol red.

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[1] Goldring, W., Clarke, R. W., and Smith, H. W., *J. Clin. Invest.*, 1936, **15**, 221.

In a few subjects observed early in the course of the disease the phenol red/inulin clearance ratio has so far exceeded the normal range as to suggest a dissociation of glomerular and tubular function of another nature. One subject, first observed early in an initial attack of acute glomerular nephritis, showed an inulin clearance of 72%, a phenol red clearance of 156%, and a phenol red/inulin clearance ratio of 210% of our average normal figures. During the next 3 months the inulin clearance rose to 109% while the phenol red clearance fell to 132%, so that the ratio decreased to 119% of normal. In interpreting this phenomenon it must be recognized that, physiologically or functionally, the phenol red clearance might rise to excessively high absolute values because of an increase in the specific capacity of the tubules to excrete the dye, or an increase in the quantity of plasma presented to the tubules. The latter circumstance might arise from renal hyperemia due to gross delivery of an increased quantity of blood to the kidneys, or from dilatation of either the afferent or efferent glomerular arterioles, or both. On the other hand, structural alterations in the kidney might increase the phenol red/inulin clearance ratio, as when the permeability of the glomerular membranes is reduced without obstruction to blood flow, or when some glomeruli have been obliterated and a blood supply to the tubules has been reëstablished either by channalization of the fibrosed glomeruli or by new extraglomerular routes. There is at the moment no evidence that will permit a final selection from these possibilities. Since dilatation of the efferent glomerular arterioles would tend not only to increase the phenol red clearance by permitting an increase in renal blood flow, but also to decrease the inulin clearance by reducing the effective glomerular filtration pressure, this functional disturbance could explain the observations on the subject mentioned above. Whether or not efferent dilatation is the only factor involved, our observations suggest that renal hyperemia may exist during a phase of acute glomerular nephritis.

Evidences of reversible renal ischemia have been obtained in an exacerbation of chronic disease during the period of subsidence. Further discussion of this and other aspects of the problem are postponed in the hope that information may be gained on the relative importance of reversible functional as compared to irreversible structural changes in renal activity at various stages of the disease.

9501

Further Investigations of the Effect of Tissues on Autonomic Drugs.

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In previous papers[1, 2] it was established that epinephrine and acetylcholine are destroyed by tissues (in this case the scales of *Fundulus heteroclitus*); it was found also that 50% deuterium oxide inhibits this destruction. Using the same technique and method of recording as previously, the destructive effect of the same tissues has now been investigated on atropine, pilocarpine, physostigmine and mecholyl.

Three kinds of solutions were made in each case: (1) solution of the drugs used, (2) the same solution + fish scales, and (3) the same solution + fish scales + 50% deuterium oxide. All solutions were tested 4 times: immediately after they had been made, 12 hours later, 24 hours later, and 48 hours later. The test objects were melanophores of isolated scales of *Fundulus heteroclitus* freshly removed from the fish.

A. Atropine. One-half percent solution of atropine sulphate was used; in this concentration, atropine fully expands the melanophores. The solutions of atropine sulphate to which fish scales were added showed a much slighter effect after 12 hours. After 24 and 48 hours the same solutions were ineffective. If, however, 50% deuterium oxide were added together with the scales, the atropine solu-

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[1] Barbour, H. G., and Bogdanovitch, S. B., *J. Pharm. and Exp. Therap.*, 1937. In press.

[2] Bogdanovitch, S. B., and Barbour, H. G., *J. Pharm. and Exp. Therap.*, 1937. In press.

tion showed practically no change in effect on the melanophores after 12, 24, or 48 hours. Control solutions (pure one-half percent atropine sulphate) also showed no difference in effect during the whole experiment.

B. Pilocarpine. One-half percent solution of pilocarpine chloride was used; in this concentration pilocarpine fully expands the melanophores. There was no difference in the effect on fish melanophores between the solutions of pilocarpine without and with scales, even 48 hours after the scales had been added.

C. Physostigmine. One-half percent solution of physostigmine sulphate was used; in this concentration, physostigmine, too, expands the melanophores well. As with pilocarpine, there was no difference in effect between the solutions without and with scales, even 48 hours after the scales had been added.

D. Mecholyl. (Acetyl-beta-methylcholine chloride) 0.02% solution of mecholyl was used. In this concentration, mecholyl well contracts the melanophores. Mecholyl solutions also did not show significant differences in effect on the melanophores between the solutions with and without scales, even after 48 hours.

From these results it may be concluded that: (1) atropine was also destroyed by fish scales as had been epinephrine and acetylcholine. Deuterium oxide inhibits this destruction. (2) Pilocarpine, physostigmine and mecholyl resist the destructive influence of fish scales.

Effect of boiling the destructive tissues. In connection with the earlier work it was suggested that this destruction of epinephrine and acetylcholine might be due to the action of certain enzymes, and that deuterium oxide by inhibiting such enzymes protects epinephrine and acetylcholine from the destruction. To investigate further this possibility, the following experiments have been done: besides the previously used 3 kinds of solution (drug alone, drug + scales, drug + scales + deuterium oxide), a fourth kind of solution was used: drug + scales which have been previously boiled in balanced salt solution for 10 minutes at 100°C.

These experiments have been made with acetylcholine, epinephrine and atropine. The results were: scales thus boiled do not destroy any one of the 3 mentioned drugs even after 48 hours. This thermolability of the "destructive" principle in fish scales for these 3 substances is consistent with enzyme hypothesis.

9502

Bilirubin in Edema Fluids in Cardiac Decompensation.

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Meakins[1] stated that edema fluids in patients with cardiac decompensation contain no bilirubin and attempted to correlate this finding with the distribution of jaundice in patients with congestive failure. Andrews,[2] however, had previously reported the presence of appreciable amounts of bilirubin in the ascitic fluid of a patient with cardiac decompensation.

In the present studies edema fluids from various body spaces taken from 8 patients with cardiac edema were examined. Fluids and blood were withdrawn from the patient within a few minutes of each other. The method of Van den Bergh as modified by McNee and Keefer[3] was used in estimating the serum and edema fluid bilirubin contents; the protein concentrations were measured by the macrokjeldahl method according to Dyer[4] (non-protein nitrogen was subtracted from total nitrogen).

TABLE I.

Case	Type of Fluid	Protein gm. % Serum	Protein gm. % Fluid	Bilirubin mg. % Serum	Bilirubin mg. % Fluid
F.	Chest	5.64	1.47	0.6	T*
K.	Ascites	7.36	4.92	0.7	0.5
H.	Chest	7.04	1.37	0.9	0.6
W.	”	6.77	1.69	1.0	0.7
R.	Ascites	7.97	2.60	1.4	0.7
B.	Chest	6.27	1.57	1.5	0.8
G.	”	7.52	2.49	1.5	0.9
Fo.	Ascites	7.55	4.73	1.9	1.3
R.	Subcutaneous Edema	6.98	0.12	2.8	0.3

*Unmeasurable trace.

The serum bilirubin concentration ranged from 0.6 mg. % to 2.8 mg. %; that of the edema fluids varied from a trace to 1.3 mg. %. Bilirubin was found in every specimen of edema fluid; the edema fluid bilirubin content was lower than that of the serum in each instance. The data suggest that there is a correlation between the bilirubin content of the edema fluid and of the serum, and also that for a given concentration of bilirubin in the serum, its concentration in the edema fluid is in general higher, the higher the protein content of the fluid.

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[1] Meakins, J. C., *J. Clin. Invest.*, 1927, **4**, 135.

[2] Andrews, C. H., *Quart. J. Med.*, 1924, **18**, 19.

[3] McNee, J. W., and Keefer, C. S., *Brit. Med. J.*, 1925, **2**, 52.

[4] Dyer, B., *J. Chem. Soc.*, 1895, **47**, 811.

9503 P

The Distribution of Material Inoculated into the Monkey Brain.*

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When conducting experiments with poliomyelitis virus, intracerebral inoculations are used extensively since monkeys are most consistently infected by this route. However, no data are available concerning the subsequent distribution of material inoculated in this manner. A large series of experiments was performed by us, in which the resultant neutralizing action of immune serum upon the virus was tested by direct inoculation of the mixtures into the brains of monkeys. During the course of this work numerous discrepancies in the results were observed. In an effort to account for the variations, among other factors, the influence of the method used to inoculate the serum-virus mixtures into the monkey brain and the eventual course of the material from the site of inoculation following its deposition were studied. The dispersion of the inoculum was followed by the addition of India ink to the mixtures.

Experiment I. 1.5 cc. of human serum was mixed with 1.5 cc. of a Berkefeld filtrate of a 5% virus suspension. This was the usual mixture which we had used in our neutralization test experiments. To this mixture 1 cc. of India ink was added. After thorough mixing, the material was injected into the right frontal lobes of 4 monkeys *(Macacus rhesus)*, each receiving 0.25 cc., 0.5 cc., 1.0 cc., and 2.0 cc. respectively, with a tuberculin syringe carrying a ¾-inch, 26-gauge needle which was inserted through a small trephined opening made on the frontal bone approximately 1 cm. to the lateral right of the mid-line and 1 cm. anterior to the coronal suture. At the end of 2 hours, the monkeys were chloroformed and their brains and spinal cords examined at autopsy. The results are summarized in Table I.

Experiment II. A second series of 4 monkeys was inoculated in a

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* This work was aided by a grant from the President's Birthday Ball Commission for Infantile Paralysis Research.

TABLE I.

The Diffusion of Material Inoculated Intracerebrally (Right Frontal Lobe) Throughout the Central Nervous System as Evidenced by the Distribution of India Ink Contained in the Inoculum.

Monkey No.	Amount of Mixture Injected cc.	Appearance of the Central Nervous System 2 Hours after Injection
1.	0.25	There was evidence of seepage of the material from the brain substance into the subarachnoid space above the site of inoculation. Some hemorrhage at the site of inoculation was also noted. No India ink was observed on the spinal cord.
2.	0.5	When the monkey was chloroformed, leakage was observed to be still taking place externally at the site of injection. Upon flapping back the scalp, India ink was found to be deposited around the trephined opening and the surrounding area. The material had diffused throughout the surface of the brain, cerebellum, and the entire length of the spinal cord. Sections at the site of inoculation showed that the injection had probably been made directly into the lateral ventricle.
3.	1.0	This monkey had been used in a neutralization test 2 months previously and therefore, as is often observed, had a sterile necrotic abscess in the right frontal lobe at the previous site of inoculation. Upon examination it was noted that some India ink had seeped into the subarachnoid space, but the bulk of the inoculum was found to be confined to the cavity on the right side.
4.	2.0	The entire surface of the brain and cord of this animal was covered with India ink indicating the extensive seepage of the inoculum from the site of inoculation into the cerebrospinal fluid.

manner similar to those in Experiment I. In this group, 2 monkeys received 1.0 cc. and 0.25 cc. respectively, of the serum-virus-ink mixture with a ¾-inch, 26-gauge needle and 2 others were injected with 1.0 cc. and 0.25 cc, respectively, using a ½-inch, 26-gauge needle. After 2 hours, the animals were sacrificed and their brains and cords examined at autopsy. The findings are summarized in Table II.

From this study, two points become evident. Firstly, that very little of the material inoculated remains at the site of inoculation, but a large proportion of it finds its way into the cerebrospinal fluid either by seeping backward through the path of inoculation into the subarachnoid space or *via* the ventricles into the spinal canal. This seems to be most marked whenever large amounts or longer needles are employed for inoculation. Secondly, that despite all precautions to obtain uniformity in the manner of injection, the results of intracerebral inoculations are variable in so far as the ultimate distribution of the inoculum is concerned.

TABLE II.

Comparison of the Diffusion of Material Through the Central Nervous System after Intracerebral Inoculation with Needles of Two Sizes.

Monkey No.	Amount of Mixture Injected, cc.	Size of Needle, inch	Appearance of the Central Nervous System 2 Hours after Injection
1.	1.0	½	The entire surface of the right frontal lobe up to the fissure centralis was covered with India ink. No ink was observed on the opposite hemisphere nor on the spinal cord. Sections examined at the site of inoculation indicated that the inoculum had been deposited in the brain substance and the site of inoculation just below the cortex was slightly distended. Some of the material had seeped backward and entered the subarachnoid space. No ink was found in the spinal canal.
2.	0.25	½	The general appearance of the brain and cord of this animal was similar to the one above, except that there was less distension of the brain tissue at the site of inoculation.
3.	1.0	¾	The entire cerebral hemisphere opposite to the side inoculated was completely covered with India ink. Ink was also present in all of the ventricles, at the base of the brain, and on the surface of the spinal cord.
4.	0.25	¾	No ink was observed on the surface of the brain, but it was abundant on the surface of the spinal cord. Sections of the brain revealed a considerable quantity of ink in the lateral ventricle.

9504

Converse Effects of Stimulating Opposite Ends of a Cardiac Muscle Band.

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No interpretation of a given phenomenon can be considered final until verified by its application to reproduce that phenomenon. Such a demonstration has hitherto not been available to elucidate the mechanism and pathways of cardiac excitation. The problem may

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* Acknowledgment is due to the Hendricks Fund for the support of this work.

be simplified by a technical isolation of the several regions of the heart. Whereas in the standard leads, the excitation process manifests itself *in toto,* it is possible, by the application of direct contacts to the surface of the heart, to recognize the behavior of regions immediately subjacent to these contacts. Using *simultaneous* records by 3 galvanometers of equal resistance and string periodicity, one may compare the response of each locus to stimuli approaching it from one of several directions, to ascertain under what circumstances its intrinsic deflections most accurately recapitulate the normal.

In this instance 2 electrodes were placed on the left border of the heart in line with the fibre direction of the superficial bulbo-spiral muscle. Electrode "A" at the apex was connected with "B" at the base by lead I, and each with the left leg, "L.L.", as leads II and III respectively; all three being recorded simultaneously. The contour of the spontaneous beats of the heart (exposed in a dog after pentobarbital anesthesia) is shown in the cycles marked "N" in Fig. 1. Electrical stimulation of the *apex* at a point very near to contact *A* initiates a premature systole (cycle "A", Fig. 1) with intrinsic deflections having the same direction as the normal in every lead. A very different result is obtained by retrograde excitation of the muscle from a stimulus introduced at the point *B* on the base of

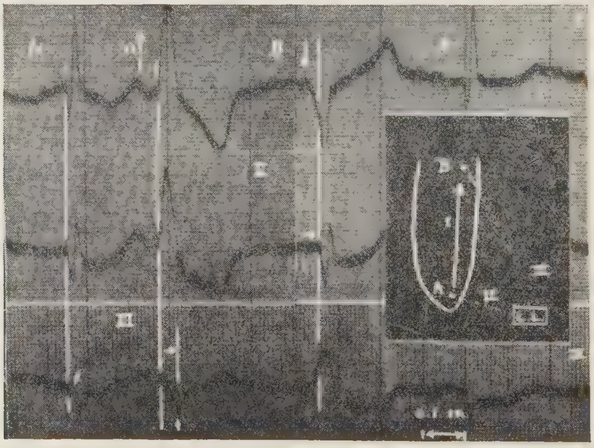

FIG. 1.

Direct lead between contacts on the surface of the heart, from the apex *A* to the base *B*, with simultaneous leads *ii* and*iii* from these points to the left leg *L.L.* Observe that the apical premature systole (A) resembles the normal cycle (N.), whereas the basal premature systole (B) in leads i and iii presents the inverse of normal. (The arrows delimit intervals for the measurement of conduction velocity, discussed elsewhere.)

the heart; both leads I and III from this region are the inverse of normal. (It is to be noted that "extrinsic" waves are lacking in each lead arising from the region of stimulation, *e. g.*, leads A I and II and B I and III. Here is evidence that only adjacent activity is recorded by this method.)

Premature beats have been recorded 41 times in 5 dogs, where direct electrodes were arranged as shown by the inset, Fig. 1. Twenty times the premature beats were excited at or near the posterior horn of the left ventricular apex and in each instance the main deflection was in the same direction as that of the spontaneous beats. Twenty-one times the premature beats were entered at the base of the heart and in each instance the main deflection was opposite in direction to that of the spontaneous beats.

Interpretation. The cephalic and caudal ends of the external fibers of the superficial bulbo-spiral muscle were stimulated alternately. Records from direct contacts on these fibers show that apical premature excitation resembles the normal sequence, whereas the product of basal stimulation is the inverse of normal.

This behavior conforms with the anatomical observation[1] that the superficial bulbo-spiral muscle is innervated by the posterior division of the left ramus of the bundle of His, entering that muscle in the posterior papillary muscle of the left ventricle and emerging to the surface in the posterior horn of the apex, to supply that muscle.

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[1] Robb, Greene, and Robb, *J. Tech. Methods and Bul. Assn. Int. Ass. Med. Mus.*, 1937, **17**, 91-92.

9505

Action of Male Sex Hormone With and Without Estrin in the Female Rat.*

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It has recently been reported that injection of male sex hormone simultaneously with estrin into normal female rats partially suppressed the characteristic histologic reaction of the anterior pituitary to estrin.[1, 2] The degree of weight increase in the pituitary and the

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* These studies were aided by a grant from the International Cancer Research Foundation made to J. B. Hamilton.

[1] Wolfe, J. M., and Hamilton, J. B., *Anat. Rec.*, 1937, **67**, 55 (suppl.).

[2] Wolfe, J. M., and Hamilton, J. B., *Endocrinology*, 1937, **21**, 603.

degree of degranulation of the chromophilic cells, together with a reduction in their relative percentages and an increase in that of the chromophobes, were more marked in the rats which received estrin alone than in those which received both estrin and male hormone. Previously, it had been demonstrated that injection of sufficient amounts of estrin into normal female rats resulted in the formation of large, active corpora lutea in the ovaries.[3-6] Estrin apparently induced such luteinization indirectly by stimulating the production or the release of a luteinizing factor by the anterior hypophysis.[5] Therefore, it was considered possible that in rats which received male hormone simultaneously with estrin there would be not only a suppressed histologic reaction of the anterior lobe to estrin but a reduction in the degree of luteinization in the ovary. In the experiments described below this assumption has been tested. The action of male hormone alone on the ovaries was also studied.

Series 1. Seventeen mature female rats received daily injections of both 500 gamma of male sex hormone† and 200 r.u. of estradiol-benzoate† for a period of 10 days while a second group of 42 females received daily injections of 200 r.u. of estrin alone for a like period. The mean pituitary weight of the rats receiving both male hormone and estrin was 11.7 mg.; that of rats receiving estrin alone was 20.4 mg. The mean ovary weight of rats receiving the combined injections was 54 mg.; the mean of those receiving estrin alone was 66 mg. The vaginal epithelium of the rats which received both male hormone and estrin was with 2 exceptions markedly mucified; these 2 exceptions showed general stratification with evidence of early mucification. The epithelium in the majority of the vaginæ of rats which were given estrin alone was stratified, with evidence of early mucification. However, a few showed stratification and in some instances cornification, while a few were markedly mucified. As a whole, vaginal mucification was much more marked in those rats which received combined injections than in those which received estrin alone.

Serial sections were cut of all ovaries studied. The 5 largest corpora lutea in each ovary were measured with a micrometer eyepiece and their average diameter calculated in millimeters. The

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[3] Hohlweg, W., *Klin. Woch.*, 1934, **13**, 93.

[4] Wolfe, J. M., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 757.

[5] Selye, H., Collip, J. B., and Thomson, D. L., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1377.

[6] Nelson, W. O., *Anat. Rec.*, 1935, **64**, 61 (suppl.).

† Testosterone-propionate and estradiol-benzoate (Progynon-B) were furnished by the Schering Corp. through the courtesy of Dr. Erwin Schwenk.

mean diameter of the corpora lutea in the ovaries of rats which received both male hormone and estrin was 1.6 mm.; the range was from 1.4 to 1.9 mm. The mean diameter of the corpora in the rats which received only estrin was 1.7 mm.; the range was from 1.4 to 2.0 mm. In 33 uninjected controls it was found that the mean diameter of the corpora lutea was 0.93 mm. These data indicate that male hormone administered with estrin at the most only slightly reduces the capacity of estrin to stimulate luteinization.

Series 2. The above experiments indicate that injection of male hormone does not possess the capacity to decrease materially luteinization induced by injection of estrin. Therefore, the capacity of testosterone-propionate to stimulate luteinization was tested. Our studies confirm and extend those of others[7, 8] who have reported that injection of male hormone increases the size of the corpora lutea.

Ten adult female rats received 500 gamma of male hormone daily for 10 days; they were killed one day after the last injection. Examination of serial sections of the ovaries revealed that in 4 animals the corpora lutea were moderately increased in size; the mean diameter was 1.4 mm. On the other hand, the corpora lutea in the ovaries of a second group of 4 of these rats were as small as those found in the ovaries of normal untreated cyclic females. The ovaries of the remaining 2 rats contained corpora lutea which were slightly increased in size. These results indicate that male sex hormone possesses the capacity to induce luteinization in some instances and suggests the possibility that the capacity of this substance, given in the amounts used in these experiments, to stimulate the formation of large active corpora lutea might be correlated with the phase of the estral cycle during which injections were initiated. This hypothesis was tested in the experiments recorded below.

Forty adult rats received daily injections of 2000 gamma of male hormone for 10 days; all were killed one day after the final injection. Previous to the beginning of injections vaginal smears were made for a period of 3 weeks and were continued throughout the injection period. In 15 rats injections were begun in estrus; in 6 the rats were in metestrus and in 19 they were in diestrus (2 days postestrus) when the injections were started.

Our data indicate that the response of the ovaries to the injections of male hormone differed, depending on the phase of the estral cycle during which injections were started. The ovaries of rats

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[7] Korenchevsky, V., Dennison, M., and Hall, K., *Biochem. J.*, 1937, **31**, 780.

[8] Nelson, W. O., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 823.

which received their first injection either in estrus or metestrus contained corpora lutea which were definitely and consistently larger than those found in the ovaries of untreated rats killed during the normal estral cycle. Individual data on the mean diameters of the corpora lutea are presented in Table I. Confirming the results of previous investigators[7, 8, 9] it was found that injection of male hormone resulted in a suppression of the estral cycle. In these 2 groups the suppression was immediate; the rats failed to exhibit the next expected estrus and remained in diestrus throughout the injection period. Histologic examination revealed that the vaginal epithelium was markedly mucified.

TABLE I.
Detailed Data on Degree of Luteinization of Ovary When Injections Were Started During Various Phases of Estral Cycle.

Aver. diam. of corpora lutea in mm.	Control	Time of Initiation of Injection		
		Estrus	Metestrus	Diestrus
.8 and below	9			9
.9	13			
1.0	4			2
1.1	7			3
1.2				2
1.3				1
1.4		6	1	1
1.5		5	2	1
1.6		1	2	
1.7		2	1	
1.8		1		
Mean diameter	.93	1.5	1.5	1.0

In the rats which received their first injection during diestrus the results were more variable. The ovaries of 9 rats contained corpora lutea which were very small (Table I). Ten rats presented corpora lutea which showed various degrees of enlargement, although only 2 were as large as those found in rats which received their first injection either in estrus or metestrus (Table I).

Histologic study showed that in general in those rats in which the corpora lutea were very small the vaginal epithelium was low with the innermost cells mucified; however, one rat with very small corpora presented a moderately thick vaginal epithelium. In those rats in which there was a definite stimulation of the corpora lutea the vaginal epithelium was thicker and more definitely mucified although the degree of mucification was seldom as great as in those rats which received their first injection either in estrus or metestrus. Vaginal

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[9] Robson, J. M., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 49.

smears of the animals receiving their first injection during diestrus revealed that with 2 exceptions, they failed to present a fully cornified smear after injections were started; in several, however, there was a mixed smear of nucleated epithelium and cornified cells on the second or third day of the injection period, the normal time of the next expected estrus. After this initial attempt at estrus in some of the rats, diestrus was prevalent throughout the remainder of the injection period. These studies indicate that testosterone-propionate possesses the capacity to stimulate luteinization when injected in sufficient amounts and that the degree of luteinization obtained is greatest when injections are initiated in estrus or metestrus. Furthermore, the degree of mucification seems in our experiments to be correlated with the degree of luteinization induced.

Studies on the pituitaries of these rats are incomplete. In the glands studied the basophiles have been completely or almost completely degranulated. In most instances the eosinophiles also presented evidence of degranulation and in some of the glands the relative level of these cells was reduced. In these latter glands there was an increase in the percentages of the chromophobes; some were enlarged and possessed enlarged negative images of the Golgi apparatus. The changes in the eosinophiles and chromophobes noted in some of these rats are in contrast to previous findings in which in normal or castrated immature rats 10 daily injections of 500 gamma of male hormone induced degranulation of the basophiles but no noteworthy changes in the eosinophiles or chromophobes.[1, 2] It should be noted that in the experiments here reported we have used a dosage of 10 daily injections of 2000 gamma of male hormone and a different type of test animal.

9506

Some Properties of the Antigonadotropic Factor.

Bernhard Zondek and Felix Sulman.

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While continuing our studies on the antigonadotropic factor of the blood[1] we examined the following properties:

1. *Thermolability.* The antigonadotropic factor was heated in a

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[1] Proc. Soc. Exp. Biol. and Med., 1937, **36**, 708, 712.

water-bath and its effectiveness was tested in rats according to the technique indicated by us.[1] Table I shows the result of examination at pH 8.0.

TABLE I.
Thermolability of the antigonadotropic factor (pH = 8.0).

Preparation	Degree of Heating	Duration of Heating	Effectiveness
Serum	56°C	1 hr.	+
”	62°C	1 ”	+
”	70°C	1 ”	+
Diluted Serum	80°C	1 ”	—
” ”	100°C (boiling)	until the appearance of the first bubble	—
Acetone Dry Powder	100°C (water bath)	1 hr.	+

Inactivation at 80° and 100° was performed while diluting 1:4, in order to avoid coagulation of the serum. Reactivation of the serum by adding fresh rabbit blood (complement) failed. The antigonadotropic factor is not destroyed by heating for one hour at 70°C.; it is destroyed, however, if heated for one hour up to 80°C. as well as by boiling up once. An acetone dry powder preparation of the antigonadotropic factor is not destroyed by one hour's heating at 100°C.

2. *Influence of pH.* Fifty mg. acetone dry powder of an antigonadotropic serum were dissolved in 4.0 cc. n/10 NaOH (pH, 13) and allowed to stand in the refrigerator for 24 hours. Then we neutralized with some drops of n HCl and added prolan. Titration in an infantile rat showed that the prolan was able to produce the HVR (anterior pituitary reactions). Consequently the antigonadotropic factor had been destroyed. In a similar way we examined the effectiveness of more diluted lyes, n/50 NaOH (pH, 12.5) and n/50 NH_4OH (pH, 11). Here the antigonadotropic factor was no longer destroyed.

In a third series 50 mg. acetone dry powder of an antigonadotropic serum were dissolved in 4.0 n/10 HCl (pH, 1) and allowed to stand in the refrigerator for 24 hours. Then we neutralized with some drops of n NaOH and added prolan. In the rat test there was no prolan effect at all; consequently the antigonadotropic factor had not been destroyed.

n/10 HCl (pH, 1) and strongly diluted lyes: n/50 NaOH (pH, 12.5) or n/50 NH_4OH (pH, 11) do not affect the antigonadotropic factor. n/10 NaOH (pH, 13), however, destroys the antigonadotropic factor.

3. *Influence of pepsin digestion.* 100 mg. of pepsin were dis-

solved in 4.0 NaCl-HCl-buffer (pH, 2.0), and 70 mg. of acetone dry powder of an antigonadotropic serum added (50 PAU*). After 24 hours in the incubator the solution was alkalized with NaOH up to pH = 9.6 (indicator: thymol blue), in order to inhibit a further pepsin effect. Then prolan was added. In the rat test we found that the prolan produced the HVR. Consequently pepsin had destroyed the antigonadotropic factor. At the same time we showed in control experiments that our pepsin in an acid solution (pH, 2.0) destroyed prolan, that in an alkaline solution (pH, 9.6) it does not affect it.

The antigonadotropic factor is destroyed by pepsin.

4. *Influence of trypsin digestion.* 100 mg. of trypsin were dissolved in 3.0 NaCl-Na_2CO_3-buffer of pH = 9.5 and 50 mg. acetone dry powder of an antigonadotropic serum were added (50 PAU). After 24 hours in the incubator the solution was centrifugalized and acidified with HCl up to pH = 3.5 (indicator: bromphenol blue), in order to avoid a further trypsin effect. Then prolan was added. The rat test showed that the prolan produced the HVR. Consequently trypsin had destroyed the antigonadotropic factor. At the same time we indicated in control experiments that our trypsin in an alkaline solution (pH, 9.5) destroyed prolan, that in an acid solution (pH, 3.5) however, it does not affect it.

The antigonadotropic factor is destroyed by trypsin.

5. *Resistance of the antigonadotropic factor to ultraviolet irradiation.* 50 mg. acetone dry powder of an antigonadotropic serum (50 PAU) were dissolved in 4.5 cc. of distilled water, hermetically shut in a quartz vessel 5 mm. deep and exposed to the light of a quartz lamp at a distance of 20 cm. for 30 minutes while being constantly cooled by an electric fan. The temperature did not exceed 40°C. The pH was 8.0. Then prolan was added. In the rat test we found that the prolan did not produce the HVR.

Ultraviolet irradiation (30 minutes, 20 cm. distance) does not affect the antigonadotropic factor.

6. *Resistance of the antigonadotropic factor to oxidation.* 100 mg. acetone dry powder of an antigonadotropic serum (100 PAU) were dissolved in 10.0 cc. of a 1% hydrogen peroxide solution and allowed to stand at room temperature for 24 hours. Then, by the catalase effect of 10 drops of blood, the oxygen was liberated, in order to

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* 1 PAU = 1 prolan anti-unit is the smallest amount of the antigonadotropic factor able to annihilate the gonadotropic effect of 1 RU of prolan. At least 10 units must be assayed in a test rat. (cf. Note 1.)

avoid action on prolan.[2] Prolan was added. The rat test revealed that the solution was still able to inactivate 100 RU of prolan. Thus the antigonadotropic factor had not been destroyed.

A 1% hydrogen peroxide solution does not affect the antigonadotropic factor.

7. *Solubility of the antigonadotropic factor in acetone.* We added to acetone in increasing concentration 25 mg. (25 PAU) respectively of dry powder of an antigonadotropic serum. Then the acetone was allowed to evaporate in the incubator for 24 hours and to the remainder distilled water was added.

Table II shows the conditions of solubility.

TABLE II.
Solubility of the Antigonadotropic Factor in Acetone.

Concentration of acetone %	Solubility of the antigonadotropic factor
10-40	easily soluble
45	slightly soluble
50-100	insoluble

The antigonadotropic factor is soluble in 40% acetone, insoluble in 50% acetone.

8. *Effect of the antigonadotropic factor upon a prolan solution masked by a 5% glycocoll solution.* It is well-known that we are able to inhibit the effect of some enzymatic substances by "masking" them with a 5% glycocoll solution. Thus we tried to protect 10 RU of prolan from the effect of the adequate amount of antigonadotropic factor by a contact of one hour with 2.5 cc. of a 5% glycocoll solution at a temperature of 37°C. The rat test revealed that no HVR (anterior pituitary reaction) occurred at all. Consequently the antigonadotropic factor exerts its effect upon the prolan-glycocoll complex in the same way as upon prolan. The reverse experiment failed as well: Antiprolan masked in a glycocoll solution maintained its effectiveness against prolan in the rat test.

The contact of prolan with a "masking" glycocoll solution does not protect it against the antagonistic effect of the antigonadotropic factor. Just the same glycocoll does not exert any influence upon the effectiveness of the antigonadotropic factor.

9. *Dialysis.* In order to ascertain the possibility of dialysis of the antigonadotropic factor we chose dialysis tubes of cellophane

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[2] v. Euler, H., and Zondek, B., *Skand. Arch. Physiol.*, 1934, **68**, 232; Zondek, B., *Hormone d. Ovariums und d. Hypophysenvorderlappens*, Vienna, J. Springer, 2nd ed., 1935, p. 242.

and cuprophane. 100 mg. = 100 PAU of an antiprolan dry powder were dissolved in distilled water and dialysis was performed for 24 hours at room temperature against 50.0 cc. aqua test. The dialysate was then inspissated in the vacuum at a temperature not exceeding 30°C. from 50.0 cc. to 5.0 cc., mixed with 10 RU of prolan and titrated in the rat test. The rats went into oestrus. Consequently the dialysate contained less than 10 PAU. The fluid in the tube in its turn revealed no loss of titre.

The antigonadotropic factor does not dialyze through cellophane and cuprophane membranes.

The preceding studies reveal considerable differences between prolan and antiprolan. Prolan is almost completely destroyed by heating for one hour to 67°C. (up to 90%)[2]; the antigonadotropic factor, however, is destroyed only at 80°C. Prolan is strongly affected (according to Evans[3]) by n/10 HCl, but never by n/10 NaOH. The antigonadotropic factor, reversely, is destroyed by n/10 NaOH, not, however, by n/10 HCl. Pepsin and trypsin digest prolan and the antigonadotropic factor in the same way. Prolan is very sensitive to ultraviolet rays,[2] it is destroyed within 15 minutes at a distance of 20 cm.[2] The antigonadotropic factor, however, is not destroyed at this distance, even if exposed to the rays for 30 minutes. Prolan is destroyed in a great part by a 1% hydrogen peroxide solution, the antigonadotropic factor is not influenced by this substance. The behavior towards glycocoll solutions is rather similar for prolan and antiprolan. The solubility in acetone is about the same for prolan and antiprolan.

As to the solubility of the antigonadotropic substance in acetone, there is a considerable difference if compared with the antithyrotropic factor. Anderson and Collip[4] point out that the antithyrotropic substance can be dissolved in 66% acetone. We, however, find the antigonadotropic factor to be soluble only up to a 40% acetone. Other differences are as follows: Eitel and Loeser[5] indicate that the antithyrotropic protective substance is to be found not only in the blood but in the spleen and the liver of the preliminarily treated animals. This statement does not apply to our antigonadotropic principle. It is to be found exclusively in the blood but never in other organs, nor in the urine.[1] The latter writers, furthermore, aver that the formation of the antithyrotropic factor is dependent upon the presence of the thyroid gland, or at least, in thyroidectomized animals, upon the simultaneous effect of the thyroidal incretion (simultaneous application of thyroxin). We found the antigonadotropic substance in the blood of castrates as well.[1] The antithyrotropic principle circulates more or less in every organism, as Collip discovered. We performed similar examinations with the antigonadotropic hormone and found that a normal serum—in our rat test—was more likely to increase than to inhibit the effect of an added prolan solution. Collip's antithyrotropic principle does not react specifically as to the species. The antigonadotropic factor, however, examined by us reacts highly specifically as to the species, it has even, to a great degree, a relatively high organ-specificity.[1]

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[3] Evans, H. M., *Mem. Univ. Calif.*, 1933, **2**, 66.

[4] Anderson, E. M., and Collip, J. B., *Lancet*, 1934, 784.

[5] Loeser, A., *Arch. Exp. Path. u. Pharm.*, 1936, **184**, 23.

Summary. In the above experiments we pointed out the following properties of the antigonadotropic factor derived from blood: It is not destroyed by one hour's heating to 70°C. It is, however, destroyed at 80°C., and by being boiled up once in diluted solutions. Heated for one hour at 100°C. in an acetone dry powder preparation it will not be destroyed. It is destroyed by pepsin, trypsin and n/10 NaOH. It is, however not affected by n/50 NaOH, n/50 NH_4OH, n/10 HCl, 1% H_2O_2-solution, ultraviolet irradiation. It is soluble in 40% acetone, insoluble in 50% acetone. It does not dialyze through cellophane and cuprophane membranes. We cannot exert any influence upon the mechanism of the prolan-antiprolan effect by "masking" with 5% glycocoll solutions. We pointed out the difference between the gonadotropic and the antigonadotropic factor, as well as between the antigonadotropic and antithyrotropic factor.

9507

Mechanism of Prolan-Antiprolan-Reaction in Simultaneous and Unsimultaneous Application of Both Active Principles.

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In previous investigations on antiprolan[1, 2] we used a certain standard method for determining the antigonadotropic effect. We chose the simultaneous injection of prolan and antiprolan after 2 hours' incubation. In the meantime we varied the time factor and investigated which differences of time could be permitted between the injection of prolan and that of antiprolan. We did not at first consider the quantitative side of the experiment, but compared qualitatively the effectiveness of large doses of antiprolan upon small doses of prolan with regard to the time within which an effect could be obtained.*

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[1] Zondek and Sulman, PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 708.

[2] *Ibid.*, 1937, **36**, 712.

The experimental technique was as follows: Infantile female rats, 3-4 weeks of age weighing about 25-30 gm. received one subcutaneous injection each of 10 or 20 RU of prolan in the left flank. Besides this 10 times as great an amount of antiprolan (100-200 PAU†) was injected either previously, subsequently or simultaneously into the neck. Sixty hours after the first prolan injection we began with the vaginal smears and 60 hours later the animals were killed and uteri and ovaries examined macroscopically. Dubious cases were examined histologically in a complete series. First we determined the smallest amount of antigonadotropic factor which, after 2 hours' contact with 10 RU of prolan in the incubator, was able to annihilate the gonadotropic effect in the infantile rat. According to our above definition,* we called this minimal amount 10 PAU.

1. In a first series we injected 10 PAU of antiprolan into the neck of infantile rats simultaneously with 10 RU of prolan into the flank. The gonadotropic effect was only incompletely impaired, *i. e.*, there remained a certain excess of prolan, sufficient to bring about proestrus in the animal and to induce a slight increase in weight of ovaries. The intraperitoneal injection also proved to be inferior to the "incubator-contact method." If we have a certain antiprolan excess, however, the separate injection of antiprolan and prolan can be used with as great a certainty as the simultaneous injection of a mixture of prolan and antiprolan after 2 hours' contact in the incubator.

2. In the following series of experiments antiprolan was injected previously to prolan and the result of the gonadotropic reaction determined (Table I).

Table I demonstrates that antiprolan is still effective 8 days after injection into the body. During this time the rats are refractory toward 10 RU of prolan. After 10 days, however, the effect of antiprolan can no longer be proved.

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* Quantitative results will be reported later.

† 1 PAU = 1 prolan anti-unit is the smallest amount of antiprolan able to annihilate the gonadotropic effect of 1 RU of prolan. At least 10 units must be assayed in a test rat.

TABLE I.
Result of the Gonadotropic Reaction After Previous Injection of Antiprolan.

Antiprolan Injected First PAU	Prolan Injected Later RU	Difference of Time	Gonadotropic Reaction HVR I, II, III
100	10	1 hr.	neg.
100	10	6 ”	”
200	20	12 ”	”
200	20	1 day	”
200	20	2 ”	”
200	20	3 ”	”
100	10	6 ”	”
100	10	8 ”	”
100	10	10 ”	pos.

We investigated further as to whether antiprolan circulates in the blood while it is still effective or is deposited in the organs. We injected 100 PAU of antiprolan subcutaneously into young rats weighing 20 gm. Sixty hours later the animal was bled completely and the circulatory system washed, starting from the heart, with 5 cc. of normal saline. Then the lavage water and the blood serum (on an average a total of 6 cc.) were injected into infantile female rats together with 10 RU of prolan. The gonadotropic reaction was negative. These results furnish proof for the assumption that the antiprolan circulates in the blood even as long as 60 hours after injection.

We examined the bodies of rats preliminarily treated with antiprolan for their content of this factor, using the extraction technique indicated by us previously.[1] We found that if the bleeding was insufficient, from the 100 PAU of antiprolan which had been injected 10 PAU could be proved even 60 hours after injection in the body of the animal. If the bleeding was sufficient even that 10% of antiprolan could not be traced. The small quantities of antiprolan which can occasionally be found in the body are probably attached to remnants of blood which remain in the organs when the bleeding is insufficient. In any case the separate extraction of the organ fractions (muscle, bones, central nervous system on the one hand and skin on the other) did not reveal any quantities of antiprolan.

3. In the third series inversely prolan was injected previously to antiprolan and the results of the gonadotropic reaction were then remarked (Table II).

Table II shows that even 12 hours after the injection of prolan we assuredly succeed in annihilating the gonadotropic reactions by means of antiprolan. Twenty-four hours later in one part of the experiment the prolan effect was completely annihilated, in another

TABLE II.
Result of the Gonadotropic Reaction in Subsequent Injection of Antiprolan.

Prolan Injected First RU	Antiprolan Injected Later PAU	Difference of Time	Gonadotropic Reaction HVR I, II, III
10	100	10 min.	neg.
10	100	30 ”	”
20	200	1 hr.	”
20	200	2 ”	”
20	200	6 ”	”
20	200	12 ”	”
10	100	24 ”	”
20	200	24 ”	Slight swelling of the follicles and proestrus
10	100	36 ”	pos.

part we found a slight prolan effect (slight swelling of the follicles and proestrus). This shows that 24 hours is exactly the space of time during which we can succeed in annihilating the gonadotropic effect by means of antiprolan.

4. In a further series we investigated whether gonadotropic reaction could be inhibited by way of the digestive tract, while separately applying prolan and antiprolan, *i. e.,* we administered antiprolan enterally. One hundred and fifty PAU of antiprolan were prepared as an acetone dry powder (60 mg.) and dissolved in 1 cc, of distilled water. One half was then given to an infantile female rat per oesophageal sound (human urethral catheter). Three hours later the second portion was given per sound. One hour after that 10 RU of prolan were injected subcutaneously. The gonadotropic reaction was positive, which means that antiprolan has no effect when applied enterally. This is in conformity with our observation[2] that antiprolan is destroyed by pepsin and trypsin. Consequently, the effect of antiprolan can be produced only in the parenteral way.

The above experiments tell us something about the mechanism of the effect of antiprolan. The peculiarities with regard to species and organ specificity of antiprolan mentioned previously[2] had given an indication that we should not have to deal here with a purely hormonal mechanism. We said at that time that this mechanism certainly did not run humorally, as *e. g.,* the insulin-adrenalin-mechanism controlling the sugar metabolism, but that the 2 active principles (prolan and antiprolan) must have a relation to one another like that of a key to the lock to which it belongs. If we could succeed in preserving the effect of antiprolan in the body for 8 days, *i. e.,* in rendering the body of the rat insusceptible to prolan, it would mean also that antiprolan does not work like a hormone, but rather as an antibody, by the help of which we are enabled to exert passive immunization for a certain period. As was shown by one of us previously[3] the antigonadotropic factor, even though it cannot be recognized by means of the usual methods for proving antibodies (precipitation and complement fixation), is very similar to the antibodies in many respects.

The fact that we succeeded in the rat in annihilating the gonadotropic effect by means of the homologous antigonadotropic factor even 24 hours after application of prolan seems to be contrary to the results of Parkes and Rowlands.[4] They succeed only one hour after cohabitation in inhibiting rupture of the follicle by the injection of heterologous antigonadotropic factor in the rabbit. The authors used an antigonadotropic serum prepared by prolonged injection of rabbits with *ox* anterior pituitary extract. If the authors had had a homologous antigonadotropic factor (against rabbit pituitaries) at their disposal, it is highly probable that they would have been successful in inhibiting the follicular rupture even after more than an hour. Besides this the gonadotropic effect is more quickly produced in rabbits than in rats. This probably explains the difference in our results.

Moreover, the experiments show that the prolan effect in the ovary of the rat is not an immediate, but a protracted one, and in the beginning still preventable. The stimulating effect of prolan A (follicle ripening hormone) upon the follicle produces irreversible changes only after 24 hours, which now proceed automatically and unavoidably lead to estrus.

Summary. (1) Antiprolan has a paralyzing effect upon prolan in separate as well as in simultaneous injection in the test rat. Nevertheless, the inhibiting effect of antiprolan is stronger if we inject a prolan-antiprolan mixture which has been allowed to stand in the incubator for 2 hours. (2) The antigonadotropic effect can be observed 8 days after the application of antiprolan (100 PAU). During this period the test animal is refractory towards prolan (10 RU). (3) Antiprolan circulates in the blood of the test rat as long as 60 hours after subcutaneous injection. It cannot be proved in the organs of the rats at that time. (4) We are able to retard the gonadotropic effect of prolan in rats up to 24 hours after the injection, if we inject antiprolan within this interval. (5) If applied enterally antiprolan has no effect whatsoever.

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[3] Sulman, F., *J. Exp. Med.*, 1937, **65**, 1.

[4] Parkes and Rowlands, *J. Physiol.*, 1936, **88**, 305.

9508

Comparative Lipid Analyses of Cortex and Medulla of Beef Adrenals.

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The lipids of the adrenal gland have been assigned to a position in the cortical region of the gland. This conclusion has been based upon histological analytical techniques, employing various lipid differentiating stains, such as osmic acid, Sudan III, Nile pink or Nile blue sulfate. The effect of polarized light on the histological sections has been used as an additional criterion. In our work, described below, attempts to correlate histological lipid analyses with purely chemical analyses have resulted in rather paradoxical findings, namely that the quantitative histochemical analysis for lipids does not agree with a chemical assay for lipids in a similar tissue. Histologically lipids are demonstrable in the cortex of the gland but not in the medulla. Yet our chemical methods have shown that both the cortex and the medulla of beef adrenals contain lipids and that they are similar in composition.

I. Comparative Analysis of Cortical and Medullary Extracts for Total Lipid Content. Fourteen beef adrenals obtained fresh from a local packing house were cleaned of all extraneous body fat and then dissected into their separate cortical (85g) and medullary (24g) portions. A sample of each tissue was extracted with a 3:1 alcohol-ether mixture as described by Bloor[1] and made up to 500 cc. volume. The extracts of cortex and medulla lipids were separately analyzed for total lipid content according to Bloor's method[1] for the microdetermination of blood lipids. In addition the lipid content in each case was determined directly by evaporating the solvent from aliquots of the extract. The data are shown in Table I.

The data show the presence of nearly equal amounts of lipid in the cortex and in the medulla, calculated as percentage of the wet gland, in contrast to the analyses by histological staining methods which indicate that most of the lipids are in the cortex. The Bloor oxidative procedure gave results much lower than those obtained by direct weight. This lack of agreement is to be expected because the Bloor method estimates only total fatty acids and cholesterol and is

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[1] Bloor, W. R., *J. Biol. Chem.*, 1928, **77**, 53.

TABLE I.
Comparative Analysis of Total Lipids of Cortex and Medulla.

	Total Lipids %
(1) Direct Method, wet basis	
Cortex	6.3
Medulla	5.6
(2) Direct Method, dry basis (calculated)	
Cortex	19.0
Medulla	28.2
(3) Bloor Oxidative Method, dry basis	
Cortex	12.3
Medulla	13.1

based on oxidation factors of characteristic blood lipids. We believe that the figures by the 2 procedures are of importance in that both show the presence of comparable amounts of lipids in the medulla and cortex.

II. Comparative Analysis of Cortical and Medullary Extracts for Differential Lipid Components. Aliquot portions of similarly prepared extracts of beef adrenal cortex and medulla were analyzed for the following lipid fractions: phospholipids according to Bloor,[2] the free and total cholesterol fractions by Okey,[3] and the total fatty acid portion as described by Man and Gildea.[4]

TABLE II.
Lipid Analysis of Cortex and Medulla.

Component	Cortex*	Medulla*
(1) Phospholipid %	3.09	2.66
(2) Total Fatty Acids %	1.76	1.46
(3) Free Cholesterol %	0.230	0.300
(4) Total Cholesterol %	0.255	0.354
(5) Combined Cholesterol (calculated) %	0.025	0.054

*All analyses on wet basis.

The results of these assays show a slight increase in phospholipids and total fatty acids in the cortex. The medulla, however, contains more cholesterol. Our results for free and combined cholesterol are lower than those usually reported; however, we have checked our methods against pure cholesterol with satisfactory agreement.

Summary. While the data above are not extensive, we believe them to be of importance in demonstrating lack of agreement between chemical findings and accepted histological technique. (1) Comparative analysis of extracts of both cortex and medulla indicates that the total lipid content of the medulla and cortex of beef adrenals is essentially the same. (2) Study of the individual lipids shows more phosphatide and fatty acids in the cortex and more total, free and combined cholesterol in the medulla.

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[2] Bloor, W. R., *J. Biol. Chem.*, 1929, **82**, 273.

[3] Okey, R., *J. Biol. Chem.*, 1930, **88**, 367.

[4] Man, E. B., and Gildea, E. F., *J. Biol. Chem.*, 1932-3, **99**, 43.

9509 P

Use of Sulfanilamide in the Treatment of Type XIV Pneumococcus Infections in Mice.

L. H. SCHMIDT. (Introduced by S. Tashiro.)

From the Christ Hospital Research Institute and the Department of Biochemistry, College of Medicine, University of Cincinnati.

Rosenthal[1] showed that sulfanilamide (*p*-aminobenzenesulfonamide), used therapeutically, prolonged the lives of mice infected with 10 to 100 M.L.D. of types I, II, and III pneumococci. Cooper,[2] independently, made a similar finding in type III pneumococcus infections. Similar experiments of our own, which had been in progress prior to these publications, confirmed Cooper's results. This observation suggested the use of such therapy in infections with other types of pneumococci—IV to XXXII—and particularly in those types for which potent antisera are not readily available. The present report concerns experiments with type XIV pneumococci; this organism causes about 16% of all pneumococcus pneumonias in children less than 12 years of age and 2.5% of such pneumonias in persons older than 12 years.[3]

A type XIV pneumococcus, freshly isolated from the bloodstream of a patient suffering from bronchopneumonia, was passed through mice until maximal virulence was obtained. This organism was only moderately virulent for mice; 0.5 cc. of a 1-10,000 dilution of an 18-hour broth culture, injected intraäbdominally, invariably killed mice within 48 hours. Forty-eight mice were injected intraäbdominally with 0.5 cc. of a 1-1000 dilution of an 18-hour broth culture of this organism; 8 of these mice were untreated controls. The remaining mice were divided into 5 groups; all received subcutaneous injections of 5 mg. of sulfanilamide* (0.5 cc. of a 1% aqueous

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[1] Rosenthal, S. M., *Public Health Reports*, 1937, **52**, 48.

[2] Cooper, F. B., Gross, P., and Mellon, R. R., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 148.

[3] Bullowa, J. G. M., *J. Clin. Invest.*, 1935, **14**, 373.

* Sulphonamid P, Burroughs, Wellcome and Co.

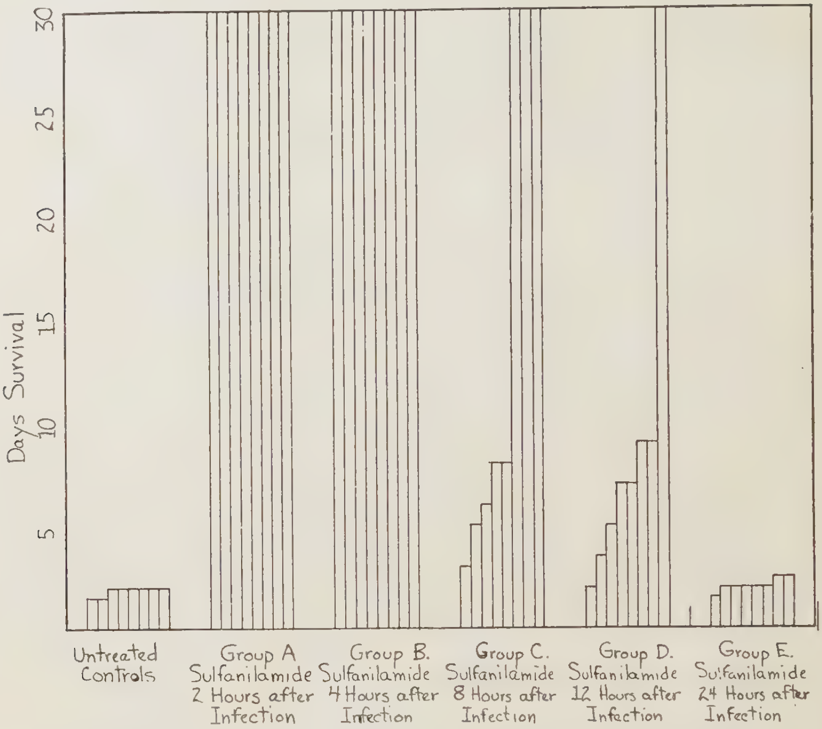

FIG. 1.

Therapeutic action of sulfanilamide against infection with multiple lethal doses of type XIV pneumococci. Therapy discontinued on 14th day.

solution) every 4 hours. This therapy was started 2 hours after infection in group A and 4, 8, 12, and 24 hours after infection in groups B, C, D, and E (Fig. 1).

When treatment was started within 4 hours of the time of infection, all of the mice survived; less protection was afforded when therapy was delayed for 8 and 12 hours although the survival time was prolonged. Therapy started 24 hours after infection was without effect on either mortality or survival time.

In another experiment 8 mice were injected with larger doses of organisms, 0.5 cc. of a 1-100 dilution of an 18-hour broth culture; 5 mg. of sulfanilamide were injected 2 hours after infection and every 4 hours thereafter for 14 days. These animals were alive and healthy 30 days later.

These results show that sulfanilamide is an effective therapeutic agent for the treatment of type XIV pneumococcus infections in mice. The effectiveness of this therapy in experimental infections suggests a study of its clinical usefulness in type XIV infections.

9510

The Hormonal Basis for "Witches' Milk."

Wm. R. Lyons.

*From the Division of Anatomy, University of California Medical School, Berkeley.**

It has been reported that about two-thirds of all babies show some breast hyperplasia for a short time after birth, and that about two-thirds of this number, regardless of sex, secrete colostrum which is usually followed by a small amount of milk.[1, 2] Topographically and histologically these breasts are miniature lactating glands,[3] and in the light of recent studies on mammary physiology such development is most likely attained through the influence of oestrin and the hypophyseal mammotropic hormone. Since oestrin is formed or stored in the placenta in considerable amounts, it was not surprising that it could be found in the blood and urine of babies for about 5 days after birth.[4-7] Abraham[8] waited until the breast swelling in new-born babies had regressed and then by injecting oestrin caused the hypertrophy and secretion to return. Brühl[5] caused mammary growth in infantile male guinea pigs by injecting 100 mouse units of oestrin daily for 18 days. These animals lactated following cessation of treatment. v. Dobszay[9] by injecting 100,000-150,000 mouse units of oestrin for 5 to 7 days into new-born babies, caused mammary growth (apparently over and above what could normally be expected) and then induced colostral secretion by injecting an hypophyseal mammotropic preparation.

Since it is possible to detect the hypophyseal mammotropic hormone in the urine of lactating women,[10] and even of normal men,†

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* This investigation was made possible by the cooperation of Dr. F. S. Smyth, Professor of Pediatrics, and his staff, and by a grant from the Board of Research of the University of California.

[1] Hoeland, H., *Monatsch. f. Geburtsh. u. Gynäk.*, 1927, **77**, 114.

[2] Fabris, S., *Pediatria*, 1927, **35**, 457.

[3] Dietrich, E. F., *Virch. Arch. f. path. Anat.*, 1927, **264**, 486.

[4] Joseph, S., *Monatschr. f. Geburtsh. u. Gynäk.*, 1929, **83**, 219.

[5] Brühl, R., *Klin. Wchnschr.*, 1929, **8**, 1766.

[6] Neumann, H. O., und Péter, F., *Z. f. Kinderh.*, 1931, **52**, 24.

[7] Winter, E. W., *Arch. f. Gynäk.*, 1932, **151**, 201.

[8] Abraham, G., *Med. Klin.*, 1930, **28**, 164.

[9] v. Dobszay, L., *Deut. Med. Wchnschr.*, 1935, **61**, 33, 1314.

[10] Lyons, W. R., and Page, E., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1049.

† In an earlier, unpublished investigation, Lyons and Vasquez obtained strong crop reactions with 100 cc. equivalents of urines from each of 7 normal men.

it seemed likely that it could also be found in babies' urine, especially in connection with the secretion of "witches' milk". The urines of 4 new-born babies (B, a female, A, C, and D, males) were, therefore, assayed for mammotropin. Case A showed breast hypertrophy at birth, and began lactating on day 3. Case C began to show slight breast hypertrophy on day 7, while B and D showed no detectable breast growth or secretion during the week of observation.

To each daily 50 cc. sample of urine an equal volume of acetone was added and this mixture stored at 0°C.‡ The acetone concentration was increased to 83% and a sufficient number of drops of 10 N HCl added to adjust the pH to 1.5-2.0 (thymol blue). After centrifuging and discarding any insoluble material, 2 volumes of acetone were added to the supernatant; then, after vigorous agitation, the sample was allowed to stand in the refrigerator overnight. After centrifuging, the supernatant was discarded, and the precipitate dried of acetone, and dissolved in 5.0 cc. of water, the pH being adjusted with NaOH to approximately 7.6 (phenol red).

The local, intradermal, crop test[10, 11] was carried out on squabs, one crop sac area being used for 3.2 cc. (= 32 cc. urine) of each specimen. This amount was given over a period of 4 days with necropsy on the 5th. When a strong reaction was obtained, smaller fractions of the remaining samples were injected into other birds. The reactions obtained were evaluated in accordance with a set of standards made by injecting a series of squabs in a similar manner with different levels of purified mammotropin prepared from sheep pituitaries. This preparation contained a micro-unit (1 M.E.D.) in 0.01 γ. Four arbitrary qualitative grades from 1 to 4 plus were established, and it was found that they could be reproduced in the majority of tests by at least 1, 4, 16 and 64 units respectively of

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Estimates made by duplicating these reactions with definite amounts of purified mammotropin showed that one might expect to find a micro-unit in from 1.5-3.6 cc. of unmodified urine. Since the completion of the work reported herein, positive crop reactions have been obtained with as little as 1.0 cc. of male urine. It is therefore suggested, that in tests for mammotropin, the urine be simply neutralized, and injected intradermally for 4 days; and, depending upon the results, concentration or dilution may be resorted to, in order to determine unitage. To detect minimal reactions, the muscle must be stripped from the reaction sites, and the inflammatory exudate must be scraped away before a reading can be properly made.

‡ Ordinarily the urines are worked-up and tested immediately, but these were unavoidably left in storage at 0°C. for 2 years. From what is known regarding the stability of mammotropin it seems unlikely that any great loss of potency occurred.

[11] Lyons, W. R., PROC. SOC. EXP. BIOL. AND MED., 1937, **35**, 645.

mammotropin.[§] Due to the small amount of urine available for each assay it was not possible to carry out a strictly quantitative assay, and therefore, the data are submitted in Table I as a qualitative contribution. The figures given represent the smallest amount of urine containing a micro-unit of mammotropin, the largest equivalent tested being 32 cc.

TABLE I.
Approximate Number of cc. of Urine Containing a Squab Micro-unit[*] of Mammotropin.

Day	Baby A	Baby B	Baby C	Baby D
1	8[†]	32	No assay	No assay
2	8[‡]	32	" "	" "
3	2[§]	<32	32	8
4	No assay	32	32	<32
5	0.5[§]	2	32	<32
6	8[§]	8	32	<32
7	32[§]	32	2[†]	8

[*] Minimal amount, which, when injected, in 4 daily doses, within the skin covering the crop sacs of squabs (one month from hatching) produces a typically positive reaction, by the 5th day, in the majority of 5 sacs from 5 different squabs.

[†] Breasts enlarged.

[‡] Colostrum secreted.

[§] Milk secreted.

As may be seen in the table, all of the babies were excreting mammotropin. In only 4 of 23 samples was it impossible to detect a micro-unit in the 32 cc. equivalent, while in lactating Baby A, a relatively high level of excretion (1 unit in 0.5 cc.) was found. The urine obtained from Baby C on the day his breasts began to enlarge (7th) contained one unit in 2 cc. Since the babies left the hospital after the first week, no further clinical observations were made. If it may be assumed that the results obtained from a random choice of 4 cases may be applied to most babies, it can be concluded that the second necessary hormone (mammotropin) is at least available, just as is the first (oestrin) for breast stimulation in the new-born. And if these 2 hormones were assayed in the same urines it would probably be shown that the relative as well as absolute quantities of each must determine whether or not lactation occurs.

Conclusions. Mammotropin was detected in the urine of 4 newborn babies. One of the babies, a boy, lactated, and his urine showed the largest amount of hormone (1 unit in 0.5 cc.). It is suggested that "witches' milk" is formed in the infantile breast after successive stimulation with oestrin and mammotropin.

[§] Although our test birds were all Silver Kings of both sexes, one month from hatching and weighed 400-450 gm., they varied considerably in their crop reactions.

9511 P

The Culture and Division Rate of *Stentor coeruleus.*

J. Gerstein. (Introduced by J. A. Dawson.)
From the Department of Biology, College of the City of New York.

Due to the difficulty of carrying on isolation cultures of *Stentor coeruleus,*[1] this protozoan has not been much used in pedigreed culture work. Hetherington[2] has recently published a culture method for this organism. He did not, however, continue the culture of Stentor for longer than 7-day periods, and also found some difficulty in keeping Stentor alive, using different types of Protozoa as food, and various media as the basis of his cultures. Beers[3, 4] has shown that *Didinium* can be cultured at a constant division rate if fed on a superabundant diet of *Paramecium.* At the suggestion and with the advice of Dr. J. A. Dawson, the writer began experiments to determine a suitable method of culturing *Stentor coeruleus* using *Blepharisma undulans* as the source of food.

A pure line of *Stentor coeruleus* was begun by isolating a single individual. As a result of 2 successive divisions four individuals were isolated and used to begin a pedigree culture of 4 lines. Each individual was placed in a separate culture dish containing 1.0 cc. of spring water (changed on June 18, 1936, to 0.8 cc.) of pH 7.2. The food consisted of a constant number of washed *Blepharisma undulans* (100-110 individuals per cc. of spring water). Isolations to fresh medium were made every 24 hours. The temperature at which the experiment was carried out was 22.1° ± .08°C. The minimum temperature during the course of the experiment was 20.1°C. and the maximum, 25.8°C.

The graph (Fig. 1) shows conclusively that the medium used is conducive to good growth of *Stentor coeruleus,* inasmuch as during the entire period the average fission rate fell below 0.5 divisions every 24 hours only 3 times, and, for the few months during which the experiment was carried on, the average rate was 6.5, with only minor fluctuations above and below. This is especially true in the last few weeks, when the technique was so perfected as to eliminate many external variables.

Although the length of time is too short for any definite conclu-

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[1] Stolte, H. A., *Arch. f. Protistenk.*, 1922, **45**, 344.

[2] Hetherington, A., *Arch. f. Protistenk.*, 1932, **76**, 118.

[3] Beers, C. D., *J. Exp. Zool.*, 1928, **51**, 121.

[4] Beers, C. D., *J. Exp. Zool.*, 1928, **51**, 485.

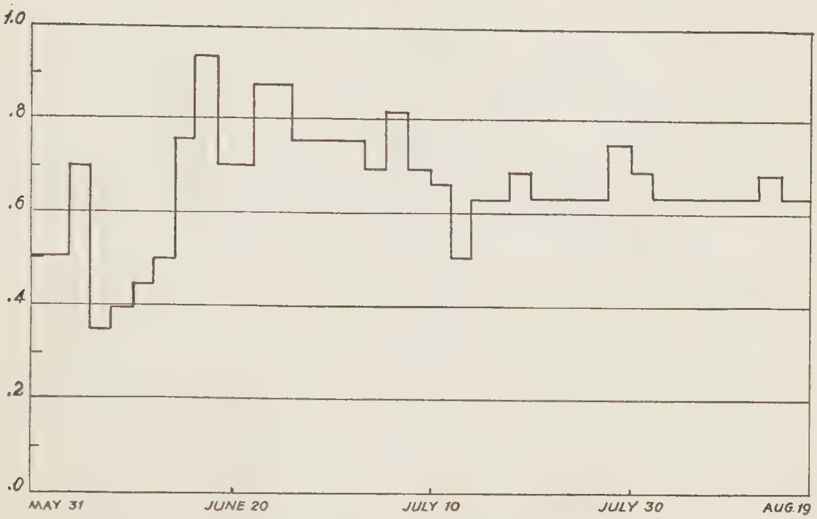

FIG. 1.

This graph shows the average fission rate for the 4 lines for 2-day periods from May 31 to August 19, 1936. The ordinates represent the average number of fissions for 2-day periods, the abscissæ represent the date.

sions concerning the question of a life cycle in *Stentor,* these results seem to point in the direction of the more recent work. Until 1925, the predominant view with regard to this question was that such a phenomenon actually existed, and that a race would die out unless a nuclear reorganization of some sort took place.[5, 6, 7] The more recent work,[3, 4, 8, 9] however, seems to point in the opposite direction, namely, that all variations are due to fluctuations in the environmental conditions, and the death of the line is due to an unfavorable environment. Since, in this experiment, the average division rate was quite constant, and the animals were apparently as healthy at the end as at the beginning, the indication is that *Blepharisma undulans* provides an adequate diet for *Stentor coeruleus.* Experiments are being carried on to ascertain whether or not a 'life cycle' exists in Stentor.

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[5] Calkins, G. N., *J. Exp. Zool.,* 1915, **19**, 225.

[6] Calkins, G. N., *Am. Nat.,* 1916, **50**, 257.

[7] Calkins, G. N., *J. Exp. Zool.,* 1919, **29**, 121.

[8] Dawson, J. A., *J. Exp. Zool.,* 1926, **46**, 345.

[9] Richards, O. W., and Dawson, J. A., *J. Gen. Physiol.,* 1927, **10**, 853.

9512 P

Changes in Electrical Potential During Oestrous Cycle of the Rat.* III. Vaginal Oestrus.

Philip V. Rogers. (Introduced by E. Allen.)

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It has been shown that the difference in electrical potential between the symphysis pubis and the vagina is directly correlated with the oestrous cycle.[1, 2, 3] A definite type of curve is obtained when the readings are plotted. After ovariectomy and hypophysectomy, the daily readings, instead of being cyclic in nature, vary in relation to each other so that the curve obtained is quite patternless. The normal cyclic type of potential is reproduced when castrate animals are given injections of theelin.

The reasons why the electrical potentials act as they do are as yet unexplained. However, an attempt is made in the following experiment to localize as far as possible the region which is responsible for the changes of electrical potential during the oestrous cycle.

Thirty-five days after ovariectomy, 16 mature rats were given intra-vaginal doses of 0.02 to 0.04 gamma of aqueous theelin. In each case the amount of hormone given was divided, the second dose being administered 24 hours after the first. About 55 hours after the first dose had been given, full cornification took place in all animals. As far as is known, the uterus takes no part in this vaginal oestrus. Electrical determinations were made on these and 7 control castrate animals every few hours throughout the duration of the experiment. Fourteen of the 16 rats coming into vaginal oestrus failed to show the rise in potential which is characteristic in those ovariectomized animals receiving subcutaneous injections of theelin. The results indicate that the potential readings are of the castrate variety where no 2 animals examined have the same type of potential curve. The increase in the difference in potential during the time of cornification in 2 out of the 14 rats may be interpreted as a part of the natural rise and fall of the potential of a castrate animal.

Preliminary results on hysterectomized animals indicate that such animals also fail to show an oestrous rise in potential when given subcutaneous injections of theelin.

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* Part of a dissertation presented for the degree of Doctor of Philosophy at Yale University.

[1] Rogers, P. V., *Anat. Rec.*, 1936, **64**, Suppl. 3, 40.

[2] Rogers, P. V., Proc. Soc. Exp. Biol. and Med., 1936, **34**, 644.

[3] Rogers, P. V., *Am. J. Physiol.*, in press.

One concludes that cornification of the vagina alone will not produce the changes in electrical potential characteristic of normal oestrus or that produced by subcutaneous injections of threshold amounts of theelin, and therefore that it is necessary to have the uterus as well as the vagina take part in the oestrous changes in order to obtain the characteristic type of oestrous potential readings.

9513 P

Potential Variations of Extremities and of Precordium in Pericarditis.

CHARLES E. KOSSMANN AND JULIUS SCHNEIDER. (Introduced by Arthur C. De Graff.)

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In pericarditis the most conspicuous abnormalities of the ventricular complex of the standard electrocardiogram are early displacement of the RS-T segment and subsequent inversion of the T-wave. It is the generally accepted view that these changes depend upon myocardial damage.[1-4]

With a view to learning more about this problem the extremity and precordial potentials[5] were studied in 8 cases of pericarditis. The etiology of the pericarditis was rheumatic fever in 3 cases, uremia in 2, tuberculosis in 1, and gonococcus infection in 2.

In neither patient with uremia did the electrocardiograms vary after development of a friction rub. At necropsy, although both showed fibrinous exudate on the visceral pericardium, neither displayed a large effusion or subepicardial myocarditis.

In the remainder, regardless of etiology, the electrocardiographic abnormalities were similar both in the standard and in the special

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[1] Wilson, F. N., Hill, I. G. W., and Johnston, F. D., *Am. Ht. J.*, 1934, **10**, 163, 176.

[2] Van der Veer, J. B., and Norris, R. F., *Am. Ht. J.*, 1937, **14**, 31.

[3] Katz, L. N., Feil, H. S., and Scott, R. W., *Am. Ht. J.*, 1929, **5**, 77.

[4] Herrmann, G., and Schwab, E. H., *Trans. Assn. Am. Phy.*, 1934, **49**, 229.

[5] Wilson, F. N., Johnston, F. D., Macleod, A. G., and Barker, P. S., *Am. Ht. J.*, 1934, **9**, 447.

leads. During the first week of acute pericarditis, when RS-T was still elevated in Leads I and II, the potential of the right arm showed a negative displacement, and of the left arm a positive displacement of this segment. There was either no deviation of the RS-T or a slight variable one in the lead from the left leg. In the case of the precordial leads this segment was negative in the curve from the right sternal margin, positive or isoelectric in the remaining leads.

During the inscription of the T-wave the potential of the right arm was negative, the potential of the left arm and that of the left leg were variable. In leads from the right side of the sternum the T-wave was always negative and more or less fused with the preceding segment. In the remaining precordial leads it was positive, and in one patient distinctly notched.

In only a few instances were the final ventricular deflections in the special electrocardiograms quantitatively outside normal limits[6] in the early stages of the disease. The progressive changes that occurred in these over a period of one or 2 weeks, however, confirmed their qualitative abnormality. These changes consisted in the gradual disappearance of RS-T displacement and in modification of the T-wave. In leads from the right arm and from the right sternal margin this deflection changed from negative to positive. In leads from the left side of the precordium it changed from positive to negative or merely became less positive. In the left arm and left leg leads T remained variable.

The myocardial injury responsible for these progressive changes in the final ventricular deflections was not apparently sufficient in degree or extent to cause important changes in the initial ventricular deflections of the extremity or precordial potentials. Considerable pericardial effusion in one case did reduce the amplitude of these deflections.

The results show first, that in pericarditis the final ventricular deflections in electrocardiograms representing the potentials of the extremities and of the precordium vary with the duration of the disease as do the same deflections in the standard leads; and second, that the QRS complex of precordial electrocardiograms is not greatly affected in acute pericarditis, a fact of practical importance in differentiating the disease from certain cases of myocardial infarction.

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[6] Kossmann, C. E., and Johnston, F. D., *Am. Ht. J.*, 1935, **10**, 925.

9514 P

The Fluorescence Spectrum of a Pigment Elaborated by the Diphtheria Bacillus.

M. O'L. CROWE. (Introduced by Augustus Wadsworth.)

From the Division of Laboratories and Research, New York State Department of Health, Albany.

A pigment which is elaborated by the diphtheria bacillus has been reported by several investigators.[1-12] The fluorescence spectrum of the diphtheria bacillus (Park-Williams No. 8) cultivated on a coagulated serum has been observed by Dhéré and Rapetti. The band lies between λ623 and 609mμ, with maximum at λ616mμ. They attribute the fluorescence to the presence of coproporphyrin.

This note records the fluorescence spectrum of the acid phase of a hydrochloric-acid-treated ether extract (made after Fischer's method) from 500 cc. of a 7-day culture filtrate of *C. diphtheriæ*, strain No. 3203,[5] grown in a modified infusion-free peptone medium containing sodium acetate and maltose. The flocculating value approximated 20 Lf. One hundred cubic centimeters of the ether extract were concentrated by evaporation to 30 cc. and then treated with 5.4% hydrochloric-acid solution in the proportion of 2 parts ether extract to one part of acid solution.

The original ether extract and, after treatment with the hydrochloric-acid solution, the ether and acid phases were examined for fluorescence when irradiated with ultraviolet light from a quartz mercury-vapor arc through a red-purple corex filter (Corning) and a 4-cm. depth of 4% copper-sulfate solution.

The ether extract from the filtrate appeared a brilliant red in

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[1] Smith, F. Campbell, *Lancet*, 1930, **218**, 529.

[2] Coulter, C. B., and Stone, F. N., *J. Gen. Physiol.*, 1931, **14**, 583.

[3] Stone, F. M., and Coulter, C. B., *J. Gen. Physiol.*, 1932, **15**, 629.

[4] Levaditi, C., *et al.*, *C. r. Soc. de biol.*, 1934, **116**, 609.

[5] Wadsworth, Augustus, Crowe, M. O'L., and Smith, L. A., *Brit. J. Exp. Path.*, 1935, **16**, 201.

[6] Dhéré, Ch., and Rapetti, L., *Bull. Acad. de Med.*, Paris, 1935, **114**, 96.

[7] Dhéré, Ch., Glücksmann, S., and Rapetti, L., *C. r. Soc. de biol.*, 1933, **114**, 1250.

[8] Ottensooser, F., Krupski, A., and Almasy, F., *Biochem. Z.*, 1935, **277**, 314.

[9] Bois, Elphège, *Recherches spectrochimiques sur quelques porphyrines animales et sur l'hématoporphyrine avec le fer.*, Fribourg, Fragnière Frères, 1927, 47 pp.

[10] Paic, Mladen, and Philippe, Marcel, *C. r. Acad. d. Sc.*, 1935, **200**, 173.

[11] Paic, Mladen, *C. r. Acad. d. Sc.*, 1936, **203**, 933.

[12] Paic, Mladen, *C. r. Acad. d. Sc.*, 1937, **204**, 298.

20 cm. depths and fluoresced with a brilliant red light. The ether phase of the treated ether extract appeared orange-red and fluoresced with a very faint grey-rose light which did not measurably affect a Wratten M plate exposed for one hour. The acid phase appeared a brilliant blue-violet and fluoresced with a brilliant red light.

The fluorescence spectrogram of the acid phase made on a Wratten M dry plate and the helium comparison spectrum are shown in Fig. 1, together with the microphotometric tracing of the fluorescence spectrogram.

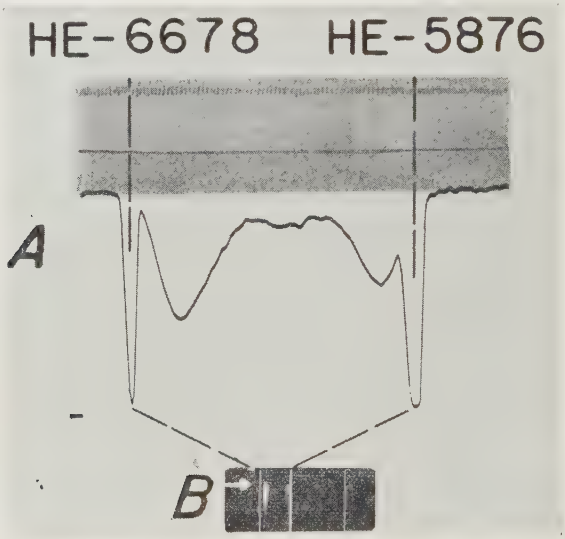

FIG. 1.

Fluorescence spectrum of the acid phase of a hydrochloric-acid-treated ether extract of a culture filtrate of *C. diphtheriæ* and the microphotometric tracing of the spectrogram.

A. Microphotometric tracing.

B. Fluorescence spectrogram with helium comparison spectrum.

Measurements of the microphotometric tracing* of the fluorescence spectrogram indicate maxima for the fluorescence bands at about λ5951, 6160, and 6502 Å. There is some evidence of "fine structure" in the bands at about λ6090 and 6280 Å. The band with the axis at λ6160 Å agrees with that observed by Dhéré and his colleagues[6, 7] in the fluorescence spectrum of diphtheria organisms. Bois's values (Atlas des spectrogrammes reproduits, p. 9)[9] for the maxima of fluorescence bands of coproporphyrin in 2 N. HCl are λ595, 620, and 653 mμ.

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* The tracing was made with a Zeiss microphotometer.

9515 P

Experimental Hypertension in Dogs by Constricting the Artery of a Single Transplanted Kidney.

CHARLES G. CHILD AND FRANK GLENN. (Introduced by Bruce Webster.)

From the Department of Surgery, New York Hospital.

Shortly after Goldblatt[1] described the production of hypertension in dogs by constriction of the renal artery, Page[2] interested himself in the relationship of the renal nerves to this type of elevated arterial pressure. He found that careful denervation of the kidney did not prevent the rise in blood pressure which follows constriction of the renal artery.

A year later, Collins,[3] employing a slightly different technique for partially occluding the renal artery, confirmed these findings. Because resection of all visible nerve filaments does not completely exclude the possibility of persistent neurogenic pathways to the kidney, the further study of the problem has been undertaken. Complete denervation has been secured by transplantation of the kidney to the pelvis, reëstablishing its blood supply by means of the femoral vessels.

These studies have been carried out on male and female dogs, weighing from 10-12 kg. Throughout the course of the experiments, blood pressure readings have been taken daily by means of the Von Leersum carotid loop. After determining the normal level of blood pressure, the left kidney has been transplanted to an intraperitoneal position within the pelvis, and its circulation reëstablished by end to end anastomosis between the renal and femoral vessels. The ureter has been left intact. After a variable period of time, usually 10 to 20 days, the normal kidney has been removed. Within the next 2 weeks the femoral artery has been constricted by means of a Goldblatt clamp.

The protocol of one animal representative of these experiments is given in Table I.

On 8 animals the artery to a single transplanted kidney has been constricted. Of these, 4 have died exhibiting terminally an elevation in the blood pressure of over 100 mm. of Hg associated with symp-

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[1] Goldblatt, H., Lynch, J., Hanzal, R. F., and Summerville, W. W., *J. Exp. Med.*, 1934, **59**, 347.

[2] Page, I. H., *Am. J. Physiol.*, 1935, **112**, 166.

[3] Collins, D. A., *Am. J. Physiol.*, 1936, **116**, 616.

TABLE I.
Animal SA-37-73. Male. Weight 12 kg.

Date 1937	B.P. mm. Hg.	Course
1/11		Von Leersum carotid loop.
1/18		Blood pressure readings started.
	140-160	Normal B.P. range for this animal.
2/8		Transplantation of left kidney. Kidney deprived of its circulation for 33 minutes.
	150-170	B.P. range after transplantation.
2/26	140-160	Right nephrectomy. B.U.N. 18.0 mg./100 cc.
3/2	140-160	Animal quiet and drowsy. B.U.N. 59.6 mg./100 cc.
3/8	140-160	Goldblatt clamp applied to the left femoral artery. B.U.N. 50.0 mg./100 cc.
3/12	240-250	
3/22	240-250	B.U.N. 25.1 mg./100 cc.
3/25	130-140	X-ray showed clamp had slipped off the artery.
4/10	130-140	Animal in good health.
5/6	130-140	” ” ” ”
5/7	130-140	Femoral artery constricted by small silver clip.
5/11	220-230	
5/19	150-160	Animal alive and in good health.

toms of an overwhelming intoxication. At autopsy, the gross and microscopic picture has paralleled that seen in normal dogs dying from too great a degree of constriction of the renal artery. In the remaining 4 animals, only transient phases of hypertension have been produced. In one animal (see table) this elevation was sustained for a period of 10 days, but, unfortunately, the clamp slipped off the artery, and the blood pressure promptly fell to normal.

Conclusion. We have concluded from these experiments that a transient hypertension can be produced by constriction of the artery to a kidney denervated by transplantation. Whether a sustained level of hypertension can be produced in the absence of the renal nerves when the artery is similarly constricted will be reported later.

9516

Effect of Relative Humidity on Gross Morphology of Certain Pathogenic Fungi.*

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In this study the effect of relative humidity under controlled air velocity and temperature on gross morphology of certain pathogenic fungi is determined.

It has been shown that ratio of constituents of mediums[1] and availability of oxygen[2] influence colony morphology. Variation in microscopic morphology with change in humidity has been studied repeatedly[3] but gross morphological changes under conditions such as used have not been recorded.

With high humidity one expects a less powerful nutrient gradient to the surface and a lesser tendency to surface growth and with low humidity the reverse. Such variation exists in nature in regions where there are deep and shallow root vegetation.[4]

To study the effect of humidity, organisms were grown on a medium producing a predominantly subsurface growth (1% d-glutamic acid, 4% dextrose, 1½% agar, pH 5.6)[5] and one producing a predominantly surface growth (Sabouraud's proof medium—1% peptone, 4% dextrose, 1½% agar, pH 5.6).

Cabinets† used for growth were placed in a room having a refrigeration control set at 70°F. An 80% relative humidity was obtained by circulating in a scrubbing tower through which air passed to the cabinets a saturated technical sodium chloride solution and a 97% by substituting tap water. Air velocity was set at 6 ft./min.

The following pathogenic fungi and 2 nonpathogenic saprophytes, *Lichthemia sp.* and *Scopulariopsis brevicaulis,* were studied: *Acho-*

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* Contribution No. 108 from the Department of Biology and Public Health, Massachusetts Institute of Technology, Cambridge, Mass.

[1] Williams, John W., *Urol. and Cut. Rev.*, 1937, **41**, 117, 198.

[2] Williams, John W. To be published.

[3] Davidson, A. M., and Gregory, P. H., *Canad. J. Res.*, 1934, **10**, 373.

[4] Salisbury, E. J., *Science Progress*, 1934-35, **29**, 409.

[5] Williams, John W., *Science*, 1936, **83**, 396; *Urol. and Cut. Rev.*, 1937, **41**, 117, 198; *Arch. Derm. and Syph.*, 1937, **36**, 581.

† I am indebted to Mr. D. G. Greenlie for aid and advice in use of the cabinets. Peptone, dextrose, agar from Difco Laboratories, Inc.; d-glutamic acid from Hoffmann-La Roche.

rion schoenleinii, Acladium castellani, Candida candida, Endodermophyton indicum, E. tropicale, Endomyces capsulatus, E. dermatitidus, Epidermophyton cruris, E. inguinale, E. rubrum, Glenospora Gammeli, Geotrichum Bachmann, Indiella americana, Microsporon Audouini, M. felineum, M. gypseum, Monosporium apiospermum, Monilia albicans, Oospora humi, Sporotrichum Schenckii, Trichophyton balcaneum, T. crateriforme, T. decalvans, T. granulosum, T. gypseum, T. gypseum-asteroides, T. gypseum-lacticolor, T. interdigitale, T. louisianicum, T. niveum, T. purpureum, T. sulfureum, Willia anomala. Observations were made on growths kept in the cabinets 30 days.

Duplicate tubes containing 10 cc. of mediums used were placed in cabinets at 80 and 97% r.h., 70°F., a.v. 3 ft./min. and 12 ft./min. The original weight of the mediums varied from 9.2 to 9.5 gm. The 38-day weight loss varied from 650 to 900 mg. Mediums at 80% r.h., a.v. 12 ft./min. showed greatest loss in weight and those at 97% r.h., 3 ft./min. least. The second highest weight loss was at 97% r.h., a.v. 3 ft./min.

In conclusion, examination of 30-day growths failed to show difference attributable to difference in humidity. Variation of nutrient gradient and evaporation was not sufficient to influence surface or subsurface tendency of growths.

9517

Dinitrophenol Hyperglycemia. II. Its Prevention by Section of Splanchnic Nerves.*

V. E. HALL AND J. O. CULVER.

From the Department of Physiology, Stanford University.

In a study of the hyperglycemia produced by 2-4 dinitrophenol (DNP), Hall, Brown and Sahyun[1] suggested that its mechanism might be the stimulation of sympathetic glycogenolytic centers of the brain stem by local cellular anoxemia or acidosis. Wishnovsky, Kane, Schlevin and Byron[2] have criticized this suggestion, on the grounds that the rise in blood sugar produced by the drug may still be demonstrated after the administration of large quantities of glucose, at a time when, as they state "the tissues are flooded with dextrose and the predominant process in the liver is glycogenesis. There is no need for active glycogenolysis." They were at a loss to explain the observed rise.

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* We wish to acknowledge the assistance of Messrs. C. A. Brown, George Fraser, and Julian Edmond during the earlier phases of the work on this problem.

[1] Hall, V. E., Brown, C. A., and Sahyun, M., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 380.

[2] Wishnovsky, M., Kane, A. P., Shlevin, E. L., and Byron, C. S., *Arch. Int. Med.*, 1935, **56**, 374.

In order to determine whether DNP hyperglycemia depends upon impulses arising in the central nervous system and causing hepatic glycogenolysis, we have cut the 3 splanchnic nerves of cats just below the diaphragm on both sides under aseptic conditions. Controls were subjected to the same procedure except that the nerves were not actually cut. After a recovery period of at least 2 weeks, the animals were anesthetized with pentobarbital (37.5 mg. per kg. intraperitoneally), allowed to rest 90 minutes so that the hyperglycemia resulting from the excitement of anesthetization might pass off, a control blood sample drawn by heart puncture and a dose of sodium 2-4 dinitrophenolate solution, adequate to yield 10 mg. per kg. of the acid was injected intramuscularly. Blood samples were drawn 30, 60 and 120 minutes after injection, and the glucose content determined by the method of Folin and Wu.[3]

The results, which appear in Table I, show that, in the controls, DNP at least doubled the blood glucose concentration, the peak usually being reached about 30 minutes after injection. However, in the cats in which the splanchnic nerves had been cut bilaterally, no

TABLE I.
Effect of Section of Splanchnic Nerves on Blood Sugar Response to DNP.

Cat No.	Initial Control mg. %	Post-injection Period 30 min. mg. %	60 min. mg. %	120 min. mg. %	Max. rise mg. %
Controls					
1	91	210	182	128	119
2	94	192	182	164	98
3	99	204	236	192	137
4	96	205	190	174	109
5	98	194	187	163	96
Aver.	96	201	195	164	112
Splanchnic nerves cut					
1	95	90	114	122	27
2	94	96	104	122	28
3	95	90	107	89	12
4	92	95	101	108	16
5	92	85	106	114	22
Aver.	94	91	106	111	21

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[3] Folin, O., and Wu, H., *J. Biol. Chem.*, 1920, **41**, 367.

such rise appears. In the first half-hour the blood sugar often falls (presumably due to accelerated glucose uptake by the tissues[4]), but thereafter shows a gradual continuous upward trend which continues throughout the period of observation. Its magnitude is much smaller than that of the rise seen in the controls. Its significance is not clear. It is not due to incomplete section of the splanchnic nerves, for this has been checked at necropsy. Fibers reaching the adrenal medulla by way of branches from the lumbar sympathetic chain may have been responsible.

It is very clear, however, that the rapid early rise in blood sugar produced by DNP depends upon the intactness of the splanchnic nerves. This fact, taken with the demonstration by Magne, Mayer and Plantefol,[5] confirmed by Hall, Field, Sahyun, Cutting, and Tainter,[6] that the blood sugar rise is accompanied by a reduction in liver glycogen content, shows clearly that the DNP hyperglycemia results at least principally from a stimulation by the drug of the sympathetic glycogenolytic mechanisms (including in this term both the direct effect of the sympathetic innervation of the liver and the glycogenolytic influence of whatever epinephrine may be secreted simultaneously). A possible additional factor may be the formation of carbohydrate from other nutrients, including fat, as has been demonstrated by Mirsky and Soskin.[7] Since this conversion was demonstrated to occur in the later phase of DNP action, it is tempting to relate it to the slow rise in blood sugar we have demonstrated in cats in which the splanchnic nerves had been cut.

Relative to the criticism which Wishnovsky and coworkers[2] have made of our concept of the mechanism of DNP hyperglycemia, it should be pointed out that all our animals have been studied in the fasting state. Under these conditions, the occurrence of sympathetically evoked hepatic glycogenolysis has been proved to our satisfaction. Wishnovsky and coworkers were studying patients who had received glucose and in whom active glycogenesis was doubtless proceeding rapidly. Nevertheless, DNP caused a further rise in blood sugar, a result fully to be expected if the drug still produced sympathetic discharge, disturbing the balance between hepatic glycogenesis and glycogenolysis in favor of the latter, so as to retard the rate of glucose uptake from the blood. Their teleological reasoning that glycogenolysis would not occur in the absence of a "need" for it is invalidated by a consideration of the hyperglycemia of asphyxia. Here, as is well known, the blood sugar is increased through the sympathetic glycogenolytic mechanism without there being any more immediate "need" for the glucose than there is following DNP administration. The close similarity of the means of production of the hyperglycemias of asphyxia and of DNP is striking. Although we[1] have shown that DNP evokes its hyperglycemia without producing an asphyxial state of the arterial blood, the possibility remains that the acceleration of the tissue metabolism in the sympathetic centers of the brain stem may result in local anoxia or acidosis and so set up the stimulus which activates the glycogenolytic mechanisms which are responsible for the hyperglycemia produced by the drug.

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[4] Ashe, W. F., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1062.

[5] Magne, H., Mayer, A., and Plantefol, L., *Ann. Physiol. Physicochim. Biol.*, 1932, **8**, 1.

[6] Hall, V. E., Field, J., Sahyun, M., Cutting, W. C., and Tainter, M. L., *Am. J. Physiol.*, 1933, **106**, 432.

[7] Mirsky, I. A., and Soskin, S., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1273.

9518 P

Influence of Insulin on Amino Acid Utilization.*

I. ARTHUR MIRSKY, SAMUEL SWADESH AND JOSEPH RANSOHOFF.

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During the course of an investigation on the endocrine control of nitrogen metabolism we found that insulin decreases the rate of protein catabolism. Our evidence suggested that this action of insulin may be due, in part, to an increased utilization of amino acids by the muscles. That insulin may have a proteinogenic action, was postulated by Wiechmann[1] and by Janney and Shapiro,[2] but neither group presented evidence to support this concept. On the other hand, Kiech and Luck[3] concluded from their studies on rats that insulin increases the rate of amino acid catabolism and inhibits the compensatory process of protein hydrolysis by which the amino acids are generated. As was subsequently stated by Daniels and Luck,[4] the possibility of an accompanying increase in the peripheral synthesis of protein from blood and tissue amino acids is not incompatible with their experimental findings.

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* Aided by the David May Fund.

[1] Wiechmann, E., *Deutsch. Arch. Klin. Med.*, 1926, **150**, 186.

[2] Janney, N. W., and Shapiro, I., *Arch. Int. Med.*, 1926, **38**, 96.

[3] Kiech, V. C., and Luck, J. M., *J. Biol. Chem.*, 1928, **78**, 257.

[4] Daniels, A. C., and Luck, J. M., *J. Biol. Chem.*, 1931, **91**, 119.

In an attempt to obtain data of a more crucial nature, we performed a series of experiments which revealed that insulin stimulates the removal of amino acids from the blood by the muscles.

Normal fasting dogs were completely eviscerated under amytal anesthesia by a modification of the method of hepatectomy described by Markowitz, Yater and Burrows.[5] The blood sugar subsequently was maintained at a normal level by a constant intravenous injection of glucose. The dose of glucose administered was 0.14 gm. per kilo of carcass weight per hour in the control groups and 0.28 gm. per kilo of carcass weight per hour in the insulin-treated group. Three groups of eviscerated dogs were studied: Group A consisted of 6 dogs receiving glucose alone; Group B, 6 dogs receiving glucose, and in addition, an intravenous injection of 600 mg. of glycine (in a 20% solution) per kilo of body weight; Group C, 6 dogs receiving glucose, glycine and in addition, an intravenous injection of 2 units insulin per kilo of body weight 15 minutes after the administration of the glycine and 0.1 unit insulin per kilo body weight at hourly intervals thereafter. Thirty minutes were allowed for the diffusion of the injected glycine before the first blood sample was drawn from an exposed branch of the femoral artery. Additional samples were drawn at 2-hourly intervals for from 4 to 6 hours. Further details will be published in a subsequent report. In this preliminary report, we wish to discuss the changes in the amino acid nitrogen of the blood which was determined by the method of Folin.[6]

The average initial amino acid nitrogen content of the arterial blood was 7.9 mg. % in Group A, 32.8 mg. % in Group B, and 36.4 mg. % in Group C. For illustrative purposes, the subsequent changes are depicted in Fig. 1 as mg. % deviation from the initial level. The data reveals that in the absence of the abdominal viscera, the amino acid nitrogen rises, presumably as a result of the cessation of deaminization consequent to hepatectomy while the hydrolysis of muscle protein continues (A). When the initial blood amino acid level is raised, the spontaneous output of amino acids from the muscles ceases and hence the amino acid level remains fairly constant (B). When insulin is administered, a significant drop in blood amino acid nitrogen occurs (C). In view of the fact that the eviscerated preparation is essentially a muscle preparation, it is probable that the amino acids removed under the stimulus of insulin are utilized by the muscles for storage or for the synthesis of pep-

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[5] Markowitz, J., Yater, W. M., and Burrows, W. H., *J. Lab. and Clin. Med.*, 1933, **18**, 127.

[6] Folin, O., *J. Biol. Chem.*, 1922, **51**, 377.

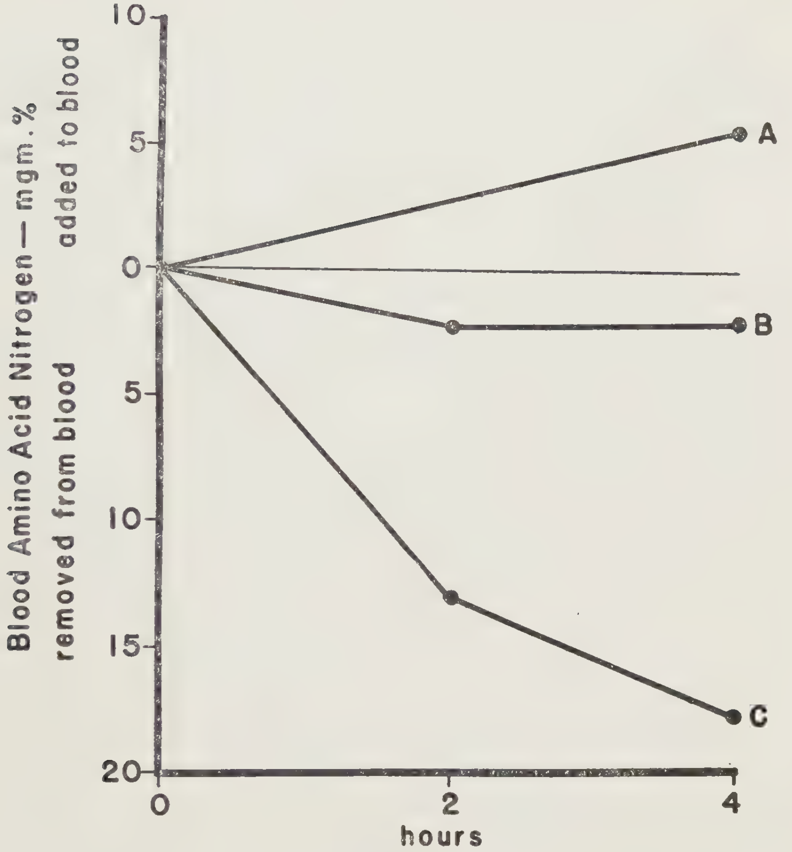

FIG. 1.

The average deviations from the initial level of the blood amino acid nitrogen in 6 eviscerated animals (A), 6 eviscerated animals which received glycine (B), and 6 eviscerated animals which received glycine plus insulin (C).

tides or proteins. The fate of these amino acids is now being investigated.

Conclusion. The spontaneous output of amino acid nitrogen by the muscles of the completely eviscerated dog is inhibited by the administration of glycine in amounts adequate to cause a hyperaminoacidemia. The administration of insulin to such animals results in an increased utilization of amino acids by the muscle. This action of insulin may be due to an increased storage of amino acids or to the synthesis of proteins in the muscles.

9519 P

Effect of Pituitary Substances on Adrenalectomized Rats.

C. J. Cavanaugh and Robert Gaunt.

From the Department of Biology, Washington Square College, New York University.

It has been shown that either the condition of pseudopregnancy or the administration of anterior pituitary substances will in certain instances extend the lives of adrenalectomized animals.[1,2,3] Among those who have used pituitary implants and extracts in adrenalectomized rats only Emery and Schwabe have found a life-sparing effect. In earlier experiments we gave large daily doses of various pituitary extracts, and of beef and rat pituitary implants (one to 2 rat pituitaries per day and similar or larger volumes of beef pituitary tissue), to partially and completely adrenalectomized rats in attempts to stimulate adrenal accessory tissue. This treatment failed to increase the percent of adrenal accessories, distinguishable by gross or histological observation, and also failed to ameliorate the symptoms of adrenal insufficiency. The substances administered were on the contrary somewhat toxic to adrenalectomized animals.

In the meantime Emery and Schwabe showed that homo-implants in smaller doses (one pituitary per week) would extend life markedly in female salt-treated adrenalectomized rats. We have confirmed their findings, using for treated and control animals the low salt diet of Weiser and Norris,[4] a diet which makes the test considerably more exacting and which has the advantage of reducing somewhat the variability in life span of adrenalectomized rats.

Adrenalectomy was performed in female rats at approximately 30 days of age. Three intramuscular implants of a whole male rat pituitary each were given in a 5-day period before adrenalectomy. Two rat pituitary implants per week were given after adrenalectomy. In all experiments litters were divided and the survival of treated animals compared to that of untreated littermate controls. Animals given implants lived twice as long as controls and during the period of their survival showed considerable weight increment (Table I). Death eventually ensued in nearly all cases despite the continuance

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[1] Rogoff, J. M., and Stewart, G. N., *Am. J. Physiol.*, 1928, **86**, 20.

[2] Swingle, W. W., Parkins, W. M., Taylor, A. R., Hays, H. W., and Morrell, J. A., *Am. J. Physiol.*, 1937, **119**, 675.

[3] Emery, F. E., and Schwabe, E. L., *Endocrinology*, 1936, **20**, 550.

[4] Weiser, R. S., and Norris, E. R., *Endocrinology*, 1936, **20**, 556.

of treatment, a fact which suggests that the value of the therapy did not lie in its stimulation of accessory cortical tissue. The treatment was non-effective under sub-optimal living conditions as demonstrated in 35 cases subjected to a low and fluctuating temperature soon after operation (due to loss of the thermostatic control of the animal room) in which both treated and control animals showed an average survival of 6 days. The replacement value of the therapy is therefore not complete.

TABLE I.
Survival of Adrenalectomized Female Rats Receiving Pituitary Implants.

No. of Animals	Treatment	Aver. Survival, days	Range, days	No. living indefinitely	Aver. wgt. diff. between operation and death
					gm.
36	Male pituitary implants	18.0	6-30	2	+12
37	None	9.7	6-15	1	— 1

Negative results have been obtained with the following materials*: 1, beef anterior lobe implants given bi-weekly in amounts approximately the same as the effective doses of rat pituitary tissue (27 cases); 2, daily doses of pregnancy urine extracts (Follutein or Antuitrin-S) in amounts varying from 15 to 50 R.U. per day (44 cases); 3, anterior pituitary extract (Squibb), found effective in cats,[2] in doses of 0.5-0.75 cc. per day (10 cases); 4, progesterone in doses of 0.15 I.U. per day, preceded in part of the cases before operation by estrogenic or pregnancy urine extracts (15 cases). The importance of dosage and optimal mode of administration is such, however, as seen in the rat implant results, that the negative findings above cannot be considered final.

Many workers have reported previously the non-beneficial effects of estrogens in adrenalectomized animals.[2, 3, 5, 6] In our experience the estrogens were distinctly toxic. A series of 17 adrenalectomized rats given a normal diet lived for an average of 7.9 days while receiving 25-50 R.U. of Progynon-B daily, whereas untreated controls lived for an average of 14.7 days. Somewhat similar results have been observed by Schacher, *et al.*,[7] and Nelson[8] has found estrin toxic in hypophysectomized male rats.

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* The authors are indebted to Dr. Gregory Stragnell, of the Schering Corporation, for the progesterone and Progynon-B used here; to Dr. J. A. Morrell, of E. R. Squibb and Sons, for the Follutein and anterior pituitary extract; and to Dr. Oliver Kamm, of Parke, Davis & Co., for the Antuitrin-S.

[5] Martin, S. J., *Am. J. Physiol.*, 1932, **100**, 180.

[6] Grollman, A., *The Adrenals*, Williams and Wilkins Co., Baltimore, 1936, p. 148.

Large doses of pituitary implants are therefore perhaps toxic rather than life-sustaining because of the high estrogen production induced. In any case the beneficial effect of pituitary therapy is not because of its estrin stimulating effects. Emery and Schwabe's interpretation that pituitary stimulation was effective because of its luteinizing effect was satisfactory for their data and for most of ours on the rat, but the non-effect of progesterone and pregnancy urine extract administration as seen here and in work on dogs,[2] and the effectiveness of pituitary extracts in castrate and male cats,[2] point to the inadequacy of this explanation for all conditions in various species.

9520

Comparison of Pituitary Gonadotropic Extract and Prolan on Ovarian and Uterine Response in Immature Rats.

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Aschheim and Zondek[1] emphasized the similarity of prolan to anterior pituitary implants or extract in its effects on the genital tract of immature mammals. Later work of other authors demonstrated several important differences, which have been summarized in recent reviews.[2, 3] Evans and Simpson[4] and Evans, Meyer, and Simpson[5] observed that prolan, in contrast to anterior pituitary extract, did not cause increasing ovarian hypertrophy with markedly increasing dosage. Similar observations were reported on placental extract by Collip, *et al.*,[6] and on extract from blood of pregnant women by Fluhmann.[7, 8] The latter also drew attention to the greater uterine hypertrophy in rats treated with prolan. Lipschutz[9] stressed the difference in luteinizing capacity between pregnancy urine and pituitary implants. This communication presents data on ovarian and uterine weights in response to varying doses of pituitary gonadotropic and pregnancy urine extracts, which can be fitted into rational curves. These define accurately the relationship of weight response to dosage and may serve as standard curves for bioassay.

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[7] Schacher, J., Browne, J. S. L., and Selye, H., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 488.

[8] Nelson, W. O., *Cold Spring Harbor Symp. Quant. Biol.*, 1937, **5**. In press.

[1] Aschheim, S., and Zondek, B., *Klin. Wschr.*, 1927, **6**, 1322.

[2] Evans, H. M., *J. A. M. A.*, 1935, **104**, 464.

[3] van Dyke, H. B., *The Physiology and Pharmacology of the Pituitary Body*, Chicago, 1936.

[4] Evans, H. M., and Simpson, M. E., *Am. J. Physiol.*, 1929, **89**, 381.

[5] Evans, H. M., Meyer, K., and Simpson, M. E., *Am. J. Physiol.*, 1932, **100**, 141.

[6] Collip, J. B., Thomson, D. L., McPhail, M. K., and Williamson, J. E., *Canad. M. A. J.*, 1931, **24**, 201.

Pituitary gonadotropic extract was prepared from acetone-dried sheep pituitary powder by the method of Wallen-Lawrence and van Dyke.[10] Further purification was carried out by fractional precipitation with alcohol and chilling, somewhat similar to the technic of Wallen-Lawrence.[11] The precipitate obtained by adding ethanol to 70% concentration and chilling at —5°C. was found to be the most potent fraction in causing ovarian hypertrophy, and was, therefore, used throughout the experiment. Prolan was prepared from pregnancy urine by adsorption with benzoic acid as described by Katzman and Doisy.[12] The crude extract was purified by precipitation by ethanol at 75% concentration. Colorimetric determination[13] on 5 mg. samples of this powder showed no detectable trace of sex hormones.

Female albino rats 21 to 22 days old from the same breed and fed on improved stock diets[14] were used. The pituitary extract was dissolved in 0.2 N acetate buffer (pH 5.3) and prolan in distilled water. Each rat received subcutaneously 3 injections of 0.5 cc. daily for 4 days, making a total of 6 cc. which contained the desired total dose of either preparation. Daily examination was made to detect vaginal opening. The rats were sacrificed approximately 120 hours after the first injection. The ovaries were carefully dissected. The uterus was cut at the level of cervix and stripped free from adventitious tissues and tubes, and fluid inside the lumen pressed out. Weighing was done on a torsion balance. The organs were sectioned for microscopic study. Controls were taken from the littermates of treated animals, and killed at the same time. Altogether

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[7] Fluhmann, C. F., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 1193.

[8] Fluhmann, C. F., *Endocrinology*, 1933, **17**, 550.

[9] Lipschutz, A., *Endocrinology*, 1935, **19**, 42.

[10] Wallen-Lawrence, Z., and van Dyke, H. B., *J. Pharm. and Exp. Ther.*, 1933, **47**, 163.

[11] Wallen-Lawrence, Z., *J. Pharm. and Exp. Ther.*, 1934, **51**, 263.

[12] Katzman, P. A., and Doisy, E. A., *J. Biol. Chem.*, 1934, **106**, 125.

[13] Wu, H., and Chou, C. Y., *Chinese J. Physiol.*, 1937, **11**, 409.

[14] Wu, H., Wan, S., and Chen, T. T., *Chinese J. Physiol.*, 1932, **6**, 295.

TABLE I.
Pituitary Gonadotropic Extract.

Dosage (mg.)	Control	.05	.10	.15	.20	.25	.50	.75	1.00
No. of animals	20	8	8	10	9	15	16	15	11
Ovarian wt. (pair)									
Mean (mg.)	13.55	14.31	14.04	13.96	16.06	16.91	28.17	58.03	73.51
Standard deviation	3.02	1.95	2.51	3.03	5.40	4.44	16.40	30.30	31.80
,, ,, of mean	0.68	0.69	0.89	0.96	1.80	1.15	4.10	7.83	9.58
Uterine wt.									
Aver. (mg.)	26.10	28.50	30.38	35.80	58.94	55.93	68.60	69.41	78.98
Standard deviation	5.38	9.87	4.86	8.14	17.11	25.10	15.56	12.28	13.20
,, ,, of mean	1.20	3.50	1.73	2.58	5.70	6.49	3.89	3.18	3.93

TABLE II.
Prolan.

Dosage (mg.)	Control	.01	.015	.02	.04	.08
No. of animals	20	10	10	10	10	10
Ovarian wt. (pair)						
Mean (mg.)	13.55	12.90	14.75	18.60	25.95	34.20
Standard deviation	3.02	2.71	3.88	3.10	6.34	6.35
,, ,, of mean	0.68	0.86	1.23	0.98	2.01	2.10
Uterine wt.						
Mean (mg.)	26.10	55.70	78.10	90.00	87.90	101.65
Standard deviation	5.38	37.61	26.61	24.40	27.90	16.72
,, ,, of mean	1.20	11.92	8.43	7.72	8.83	5.29

162 rats were included in the present experiment; 20 for control, 92 receiving pituitary extract at 8 dosage levels and 50 receiving prolan at 5 dosage levels.

The results set forth in Tables I and II are plotted in Figs. 1 and 2 for pituitary extract and prolan respectively. The trend of weight response of ovaries and uterus to increasing dosage of the 2 gonadotropic hormones corresponds satisfactorily to logistic curves. These are rational curves useful in elucidating many biochemical phenomena,[15] and have the general equation:

$$y - d = \frac{k}{1 + e^{a - bx}}$$

where y and x are variables, and d, k, a and b are constants. In our case, y represents ovarian or uterine weight, and x, dosage. The quantity d is the lower asymptote, the lower limiting value of the average ovarian or uterine weight of untreated rats, while k is the average maximum increase of the organ weight attainable with increasing dosage under the specified circumstances, thus $k + d$ giving the upper asymptote, the upper limiting value of the organ weight of

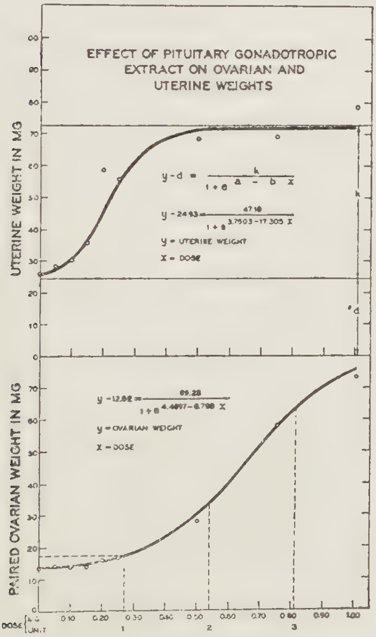

FIG. 1.

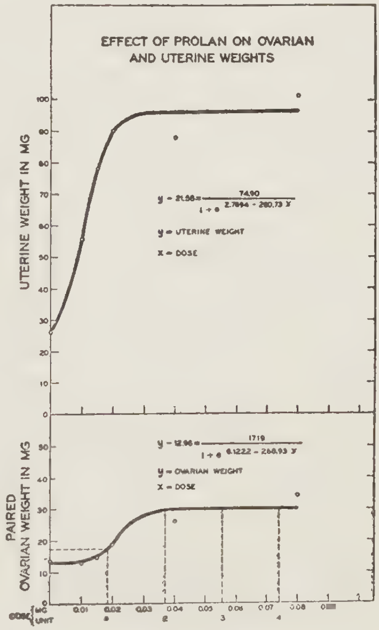

FIG. 2.

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[15] Reed, L. J., and Berkson, J., *Phy. Chem.*, 1929, **33**, 760.

treated rats. The midpoint of the curve, namely $k/2 + d$, is the point of inflection where the organ weight changes at maximum rate. The numerical values of the constants are computed by the 4-point method,[16] and given with the respective curves.

In the curves the maximum attainable weight of paired ovaries or uterus $(k + d)$ differs according to the type of extract given. With ovaries, this value is much greater for the pituitary extract-treated animals (81.10 mg.) than for prolan-treated ones (30.15 mg.), though the highest dose of pituitary extract used has not clearly established the maximum. This difference is a numerical expression of the striking contrast, observed by previous workers, between the tremendous increase in ovarian weight produced by pituitary extracts and the slight hypertrophy with prolan. On the other hand, the maximum uterine response is somewhat greater to prolan (96.48 mg.) than to pituitary extract (72.11 mg.), as observed by Fluhmann.[7]

For comparison and bioassay it is necessary to convert the weight of both pituitary extract and prolan to units of potency. A unit may logically be defined as that amount of either extract which causes a significant increase in the *mean weight* of paired ovaries of a group of animals over the control. Statistically, a difference between 2 means is considered significant when it is 3 times the square root of the sum of the squares of the standard deviations of the 2 means. When the standard deviation of the mean of the control group alone is taken, we consider an increase as significant when the mean weight of paired ovaries of the treated group exceeds the control weight by 4 times the square root of twice the square of the standard deviation of the mean of the control group. Thus in the case of paired ovaries, we have $13.55 + 4\sqrt{2 \times (0.68)^2} = 13.55 + 3.85 = 17.40$ mg., this being taken as the mean ovarian weight corresponding to one unit. Reading from the respective curves gives 0.27 mg. of pituitary extract and 0.0185 mg. of prolan as equivalent to one unit.

In terms of units as defined the ovarian weight reaches the maximum more quickly in response to prolan (2 units) than to pituitary extracts (4 units). This is also true of uterine weight. The increment of ovarian weight per unit of pituitary extract at the steep portion of the curve is much greater than that with prolan, but the corresponding increment of uterine weight is smaller.

As recorded in Table III, the 2 gonadotropic extracts differ in their capacity to provoke vaginal opening and corpus luteum for-

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[16] Pearl, R., and Reed, L. J., *Proc. Nat. Acad. Sc.*, 1920, **6**, 275.

TABLE III.
Frequencies of Opening of Vagina and Formation of Corpus Luteum.

	Pituitary gonadotropic extract						Prolan				
Dosage mg.	Total No. of rats	Opening of vagina		Corpus luteum		Dosage mg.	Total No. of rats	Opening of vagina		Corpus luteum	
		No.	%	No.	%			No.	%	No.	%
Control	20	0	0	0	0	Control	20	0	0	0	0
.05	8	1	12.5	0	0	.01	10	0	0	3	30
.10	8	1	12.5	0	0	.015	10	5	50	8	80
.15	10	2	20.0	2	20.0	.02	10	9	90	9	90
.20	9	4	44.4	1	11.1	.04	10	10	100	10	100
.25	15	9	60.0	2	13.3	.08	10	10	100	10	100
.50	16	15	93.8	8	50.0						
.75	15	15	100.0	14	93.3						
1.00	11	11	100.0	9	81.8						

mation. Prolan has greater luteinizing activity, while the pituitary extract tends to cause earlier opening of the vagina.

From the above observations, the ovarian weight curve seems more satisfactory for assay of pituitary gonadotropic extract than of prolan. However, the applicability of the curves for this purpose remains to be demonstrated. The great variability of uterine response renders its curve unsatisfactory for assay.

Summary. This study demonstrates that the function of both ovarian and uterine weight response to pituitary extract and to prolan follows the same type of curve, namely, logistic curves. However, they differ from each other markedly in the numerical values of their constants, among which the value of $d + k$ is the most noteworthy. This defines the average maximum weight response attainable under the specified conditions. It is much higher in the case of ovaries, but lower in the case of uterus when pituitary extract is given, compared with prolan. The ovarian weight curve with pituitary extract may be of interest in assay.

We wish to express our thanks to Dr. H. B. van Dyke for suggestions in preparing pituitary gonadotropic extracts, to Dr. I. C. Yuan and Mr. C. S. Hsieh for help in mathematical analysis, and to Mr. C. Y. Chou for the chemical determination of sex hormones in prolan.

9521 P

Growth of Ultracentrifuged Cells in Tissue Culture.*

T. J. MACDOUGALD, H. W. BEAMS AND R. L. KING. (Introduced by J. H. Bodine.)

From the Department of Zoology, State University of Iowa.

It has recently been shown that the eggs of *Ascaris suum* and *A. megalocephala* (Beams and King[1, 2]) Fucus eggs (Beams[3]), cancer cells of rat (Guyer and Claus[4]) and the cells of the adrenal cortex

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* Aided by grant from the Rockefeller Foundation for work on Cellular Biology.

[1] Beams, H. W., and King, R. L., *Science*, 1936, **84**, 138.

[2] Beams, H. W., and King, R. L., *Biol. Bull.* In press.

[3] Beams, H. W., *J. Mar. Biol. Assn.*, 1937, **21**, 571.

[4] Guyer, M. F., and Claus, P. E., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 468.

of rat (Dornfeld[5]) are not killed when subjected to very great centrifugal force. The significance of these experiments rests in the fact that the behavior of protoplasm to high speed centrifuging seems to differ markedly from that of many non-living colloidal systems (Svedberg[6, 7]).

In an effort to extend further these studies on the effect of ultracentrifuging upon protoplasm, the tissue culture method has been employed here. Cultures were made of embryonic chick hearts (of 9 days' incubation) by the usual Carrel hanging drop technique; 4 parts chick plasma and 3 parts embryonic extract being used as medium. The extract was in every case centrifuged twice, in order that no confusion between growth from the explanted material and growth from tissue fragments introduced along with the extract should arise. After the heart was removed, the ventricle was divided into 2 parts, one of which was placed in Tyrode solution in the rotor of the ultracentrifuge where it was centrifuged at either 150,000 or 400,000 times gravity for one-half hour; the other half being left standing in Tyrode solution at room temperature. Contamination of the experimental material was avoided by handling the sterilized rotor with a large hemostat. Cultures of both experimental and control tissue were then made at nearly the same time under identical conditions.

One hundred percent growth was obtained in both control and experimental cultures and although no quantitative determinations of actual growth were made, no difference was observed in the amount and rate of growth in the centrifuged and control material. Not only was normal mitosis apparent in the cells of centrifuged tissue, but fragments which had been centrifuged at 400,000 times gravity for one-half hour continued to pulsate up to the time of fixing (48 hours after explantation).

This study furnishes additional evidence for the fact that protoplasm is capable of withstanding very great centrifugal forces. Separation of its essential colloidal elements apparently does not take place, differing here from many non-living colloidal solutions in which separation has been beautifully demonstrated by Svedberg and others. Forces, perhaps electrostatic in nature, acting in protoplasm are possibly responsible for the failure of its structure to break down and its components to become subsequently stratified in such strong centrifugal fields.

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[5] Dornfeld, E. J., *Science*, 1937, **85**, 563.

[6] Svedberg, The., *Colloid Chemistry*, 1928, New York.

[7] Svedberg, The., *Science*, 1934, **79**, 327.

9522 P

Action of Urea, Indican, and Phenol on Red Cell Hemolysis.

S. R. Townsend* and M. Pijoan.† (Introduced by Henry A. Christian.)

From the Medical and Surgical Clinics of the Peter Bent Brigham Hospital, Boston, Mass.

In chronic glomerulonephritis usually there is a progressive normocytic anemia which becomes manifest as renal insufficiency progresses and increases with the elevation of nitrogen retention.[1] There is, furthermore, no apparent lack of active blood-forming tissue, nor has there been any evidence presented to suggest that blood loss or increased hemolysis may play a rôle.[1, 2]

With the progress of renal insufficiency in glomerulonephritis there is an accretion of various chemical constituents in the blood plasma. The increase of certain of these, namely, urea and indican, is generally accepted, and certain studies on the blood phenol indicate that this substance likewise is increased.[3] It occurred to us that these retained substances in particular may play a rôle in the anemia of nephritis, and that they may cause hemolysis of such a degree as is not detectable by the ordinary tests usually employed for the determination of increased fragility.

Herrald and Pijoan[4] investigating the behavior of erythrocytes in a hemolytic system using standardized saponin as the lytic agent, demonstrated that the red cells of nephritic patients are more resistant than those of normal subjects to saponin hemolysis. However, they were unable to offer any adequate explanation for this phenomenon. Suspecting that indican, phenol and urea might play a rôle in hemolysis, we decided to investigate this problem using the method described by Herrald and Pijoan. Our plan was to compare the saponin hemolysis curves of the red cells of uremic patients with red cells from normal subjects, and then to add proportionally the same amounts in their equivalent proportion of urea, indican and phenol to red cells of normal subjects with and without saponin.

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* Research Fellow in Medicine.

† Harvey Cushing Fellow in Surgery.

[1] Townsend, S. R., Massie, E., and Lyons, R. H., *Am. J. Med. Sc.*, 1937. In press.

[2] Brown, G. E., and Roth, G. M., *Arch. Int. Med.*, 1922, **30**, 819.

[3] Townsend, S. R. Unpublished data.

[4] Herrald, F. J., and Pijoan, M., *Am. J. Clin. Path.*, 1937. In press.

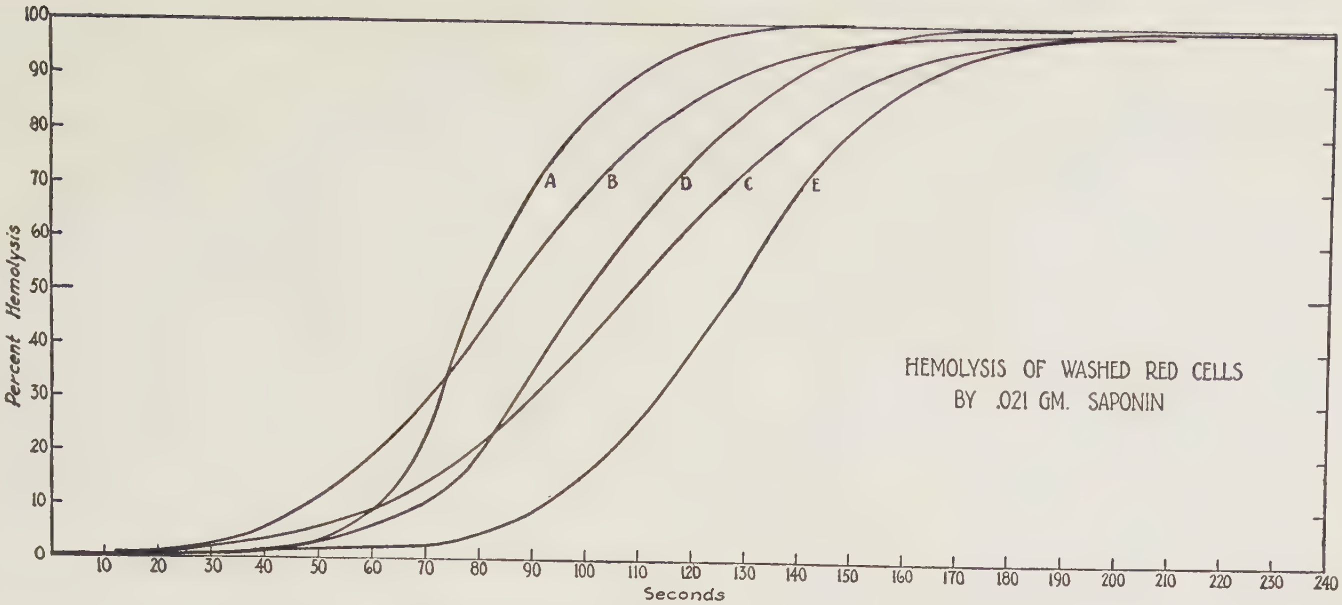

FIG. 1.

A. Hemolysis of washed normal red cells.

B. Hemolysis of washed red cells from a patient with chronic glomerulonephritis in uremia (Patient 1).

C. Hemolysis of washed red cells from a patient with chronic glomerulonephritis in uremia (Patient 2).

D. Addition to hemolytic system of normal washed red cells of equivalents in blood of 60 mg. % urea, 3 mg. % indican and 5 mg. % phenol.

E. Addition to hemolytic system of normal washed red cells of equivalents in blood of 120 mg. % urea, 3 mg. % indican and 5 mg. % phenol.

Red cells from uremic patients with the exception of those in the extreme terminal stage were found to hemolyze more slowly in saponin systems than normal cells, which is in agreement with the previous study.[4] In this investigation certain quantities of indican, urea and phenol were added individually and collectively to a suspension of known amounts of washed normal red cells in saline and were found to have no hemolytic activity whatsoever in their proportion found in the plasma. Furthermore, as can be seen from Fig. 1, urea, phenol and indican, added to systems of red cells in plasma, definitely inhibited saponin hemolysis and reproduced curves which could be compared to those obtained by Herrald and Pijoan for nephritic blood.

In light of these test tube experiments we are led to the belief that in the anemia of nephritis, which develops with the manifestation of renal insufficiency, there is no increased hemolysis of the red cells by urea, indican and phenol, and that these substances inhibit the action of a standard hemolysin.

9523

Effect of Urea Upon Growth of Fibroblasts from Cardiac Explants in Tissue Culture.

DUNCAN C. HETHERINGTON AND MARY E. SHIPP. (Introduced by F. H. Swett.)

From the Department of Anatomy, Duke University School of Medicine.

It has been found recently that allantoin, a purine derivative occurring in maggots' secretions, stimulated the healing process in deep purulent wounds.[1] Another derivative, urea, was discovered to be more effective in bringing about the same results.[2] It was thought that, since urea may be obtained from allantoin by hydrolysis, the growth promoting effect of allantoin might be due to the urea and not to allantoin itself.

Fibroblasts from cardiac explants in tissue culture grew more abundantly in the presence of 0.5% allantoin than those in control cultures; although the growth increase was not sufficiently great to be of real significance.[3] It was deemed of interest to discover

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[1] Robinson, William, *J. Bone and Joint Surg.*, 1935, **17**, 267.

[2] Robinson, William, *Am. J. Surg.*, 1936, **33**, 192.

[3] Shipp, Mary E., and Hetherington, Duncan C., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 180.

TABLE I.

	Urea 0.5% Series								Control Series				
Days Growth	Mean Total Area, sq.mm.	Probable Error of Mean	Standard Deviation	Total Growth, %	Total Death, %	D—Difference of Means	σ_D—Standard Error of Difference of Means	*Significant difference of Growth, $D > 3\sigma_D$	Mean Total Area, sq.mm.	Probable Error of Mean	Standard Deviation	Total Growth, %	Total Death, %
0	0.89	±.02	± .36	0	4	0	.050	$0 < .15$	0.89	±.04	± .37	0	1
1	1.13	±.02	± .33	27	4	.04	.052	$.04 < .16$	1.17	±.03	± .41	34	1
2	2.39	±.05	± .85	168	6	.30	.121	$.30 < .36$	2.09	±.05	± .88	123	2
3	4.26	±.13	±1.97	379	8	.62	.273	$.62 < .81$	3.64	±.13	±1.92	309	6
4	5.16	±.20	±2.82	468	9	.20	.449	$.20 < 1.35$	4.96	±.19	±2.97	343	11
5	6.49	±.22	±3.37	629	19	.21	.469	$.21 < 1.41$	6.28	±.22	±3.36	550	14
6	8.08	±.29	±4.52	808	21	.11	.640	$.11 < 1.92$	7.97	±.32	±4.83	795	17
7	9.66	±.33	±5.11	985	25	.41	.900	$.41 < 2.70$	10.07	±.42	±6.36	1031	21
8	11.29	±.43	±6.63	1169	26	.90	.895	$.90 < 2.68$	12.09	±.51	±7.63	1253	22
9	11.85	±.47	±7.21	1231	32	.71	1.044	$.71 < 3.13$	12.56	±.53	±7.92	1311	33
10	11.93	±.47	±7.11	1241	37	.83	1.030	$.83 < 3.09$	12.75	±.53	±7.77	1332	35

*Significant difference of growth was determined for any given day from the following formulæ:

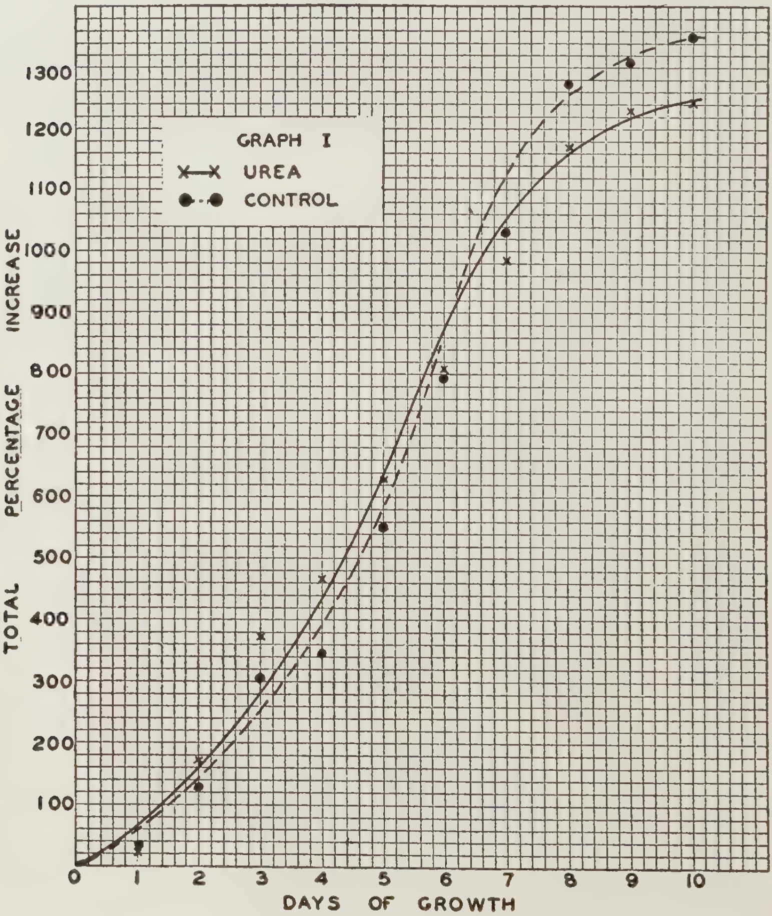
$$\sigma_D = \sqrt{\frac{\sigma_1^2}{N_1} + \frac{\sigma_2^2}{N_2}} \text{ and } D > 3\sigma_D$$

Where for any day σ_1 and σ_2 are the standard deviations of the control and urea series respectively and N_1 and N_2 the number of cultures; ''D'' represents the difference of the means and σ_D is the standard error of the difference.[4]

[4] Mills, *Statistical Methods*, H. Holt and Co., 1930, p. 558.

whether the simpler product, urea, would increase the growth of fibroblasts to a greater extent than did the allantoin and to this end the present experiment was devised and conducted.

All cultures were made by the cover-slip-hanging-drop method with the observance of aseptic precautions. All water was triply distilled in an all-glass pyrex apparatus. The plasma was obtained by centrifuging blood drawn, without the use of an anti-coagulant, from the wing veins of young hens. The embryo juice was made by extracting 7-day chick embryos in Tyrode solution (pH 7.5) containing 0.25% dextrose. For the test series, urea, in quantities sufficient for a final dilution of 0.5%, was added to an aliquot part of an adjusted Tyrode solution before the extraction of the embryos. This concentration of urea was chosen because it was the same as that of



allantoin used in a previous experiment[3] and further because the 2% solution, as used in clinical treatment of deep wounds[2] was too toxic for the cultures. Heart tissue of 7-day chick embryos was planted in a mixture of equal parts of plasma and of embryo juice, the latter containing the urea. An equal number of controls using the same plasma, stock embryo juice and embryo hearts was planted at the same time as each series of urea cultures. All cultures were incubated at 37°C. and a daily record was kept on each culture until death. With the use of a delineascope and planimeter the area of the original explant and the areas of the daily outgrowths of each of the 335 cultures were measured. One hundred of the control cultures and one hundred of the experimental cultures lived for 10 days or more and were used for statistical study of the daily growth, because it was found that the majority had reached their maximum growth before the tenth day and also because the results could be based upon and compiled from the same hundred cultures for each day. (Table I.)

Neither the control cultures nor the experimental series showed any significant differences in the behavior or structure of the cells. The difference in death rate for the 2 series of cultures (Table I) indicates that the presence of urea had no detrimental effect in these experiments. Graph I was constructed from statistical data of Table I and it may be seen that the growth curve of the experimental series indicates a wholly insignificant increase over that of the control from the second through the sixth day. Thereafter the control curve rises above the experimental one.

It has been reported that urea in suitable dilutions produces proliferation of capillaries by sprouting.[5] Since this compound does not bring about a significant stimulation of the growth of fibroblasts from cardiac explants in tissue culture, it is suggested that the hastened healing observed in deep, infected wounds following irrigation with urea solutions may be attributed to changes in the capillary bed and the associated effects therefrom upon the granulation tissue.

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[5] Abel, Richard, Abstract, *Anat. Rec.*, Supplement No. 3, 1937, **67**, 1.

9524

Effect of Testosterone Injections upon the Course of Pregnancy in Unoperated and in Castrated Rats.

E. Scipiades. (Introduced by Carl G. Hartman.)

From the Department of Embryology, Carnegie Institution of Washington, Baltimore.

Since the male sex hormone testosterone* has become generally available, the current literature abounds in reports of its action in both the male and the female vertebrate. Among the various androgenic substances thus far isolated, testosterone most nearly resembles progesterone, hence in its action upon the reproductive tract it might also be expected to simulate that ovarian hormone.

Some experiments carried out at the Carnegie Laboratory corroborated the findings of others that testosterone causes a mucification of the vaginal mucosa and no cornification, and that in the monkey growth of the mammary tree and production of alveoli are stimulated by it. Markee (unpublished), working in the same laboratory in 1935-6, noted that testosterone, injected with estrin (Amniotin Squibb), antagonized the action of the latter in producing a reduction in the size of intraocular endometrial transplants, an effect which progesterone does not bring about. Hartman found that testosterone, like progesterone, prevented the appearance of an expected menstruation in the monkey, but, unlike progesterone, failed to cause any progravid changes in the endometrium.

From these experimentally determined facts it seemed theoretically possible that the injection of testosterone into a pregnant animal might well prevent parturition or abortion, even though the androgen might not replace progestin in other respects. Thus, in the rat, the abortion which always occurs after castration of the pregnant female, might well be prevented by testosterone and the injections so adjusted that delivery occurred at term. Or again, injection of the androgen towards the end of a normal pregnancy might be expected to postpone parturition, as happens with progesterone (King).

The results of 18 of the experiments carried out along these lines are recorded in Table I, from which the following conclusions may be deduced:

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* It is a pleasure to record my thanks and that of the Department of Embryology, whose guest I have been during the past year, to the Ciba Company for a generous supply of Testosterone and Testosterone Proprionate manufactured by them.

TABLE I.

Animal No.	Date of Fertile Coitus	Date of Castration	Injections	Quantity inj. (mg.) daily*	Date of delivery	Days of Pregnancy	Condition of Fetuses
2	Jan. 29	—	Feb. 12-25	5	Feb. 26	28	Resorption and dead fetuses
1	Feb. 13	—	Mar. 5-9	5	Mar. 9	24	Living
5	Jan. 27	—	Feb. 10-21	5	Feb. 25	29	Resorption and dead fetuses
8	,, 29	—	,, 12-25	5	,, 25	27	,, ,, ,, ,,
4	,, 28	Feb. 15	,, 11-19	5	,, 19	22	,, ,, ,, ,,
7	Jan. 30	,, 15	,, 13-19	5	,, 19	20	,, ,, ,, ,,
11	,, 28	,, 15	,, 13-19	5	,, 19	22	,, ,, ,, ,,
9	Feb. 23	Mar. 11	Mar. 10-20	5	Mar. 20†	25	Living
14	Mar. 9	—	,, 27-30	5	Apr. 2	24	8 living, 4 dead fetuses
6	,, 10	—	,, 27-31	5	,, 3	24	3 living, 3 dead fetuses
22	,, 10	Mar. 27	,, 27-30	5	,, 1	22	Dead fetuses
16	,, 9	—	,, 27-30	2.5	,, 3	25	3 dead
19	,, 9	—	,, 27-30	2.0	,, 2	24	5 living, 3 dead fetuses
15	,, 20	Apr. 2	Apr. 2-7	2.0	,, 12	23	Resorption and dead fetuses
2§	Apr. 7	,, 22	,, 22-27	1.0	,, 29	22	,, ,, ,, ,,
8§	,, 13	,, 27	Apr.. 27-May 1	1.0	May 5	22	Dead fetuses
10	,, 23	May 7	May 6-10	1.0	,, 14	21	,, ,,
16§	,, 21	,, 5	,, 4-9	1.0	,, 12	22	,, ,,

*The first 8 rats of the table received the free Testosterone Ciba, the last 10 Testosterone Proprionate Ciba.

†Killed Mar. 20, 3 living fetuses found in utero.

§These 3 rats were used a second time, having conceived after the previous treatment with Testosterone.

1. Testosterone prevents or postpones parturition in the non-castrated pregnant female rat. If parturition does take place it is usually much delayed over the normal 21 days of gestation, which is the average for the strain used.

2. Testosterone is injurious to the embryos, probably through deleterious effect on the endometrium. Living embryos are to be expected only if injections are made for a short time towards the end of gestation. No. 9 of the table is exceptional.

3. Testosterone prevents the abortion which follows castration in the rat. A 5-day period of injection after castration will carry the fetuses to term, but only dead fetuses are usually delivered.

4. The larger the dosage, the more deleterious is the effect on the embryos. One milligram per day is sufficient to control the tendency of the uterus to empty itself.

5. The mode of action of testosterone on the pregnant uterus is not elucidated by the results presented in the table. The gross appearance of the uterus at autopsy argues for an excessive flaccidity of the uterine musculature.

6. The injection of testosterone in the amounts used in these experiments does not necessarily render the experimental animal sterile, for Nos. 16, 8 and 2 conceived 3, 7, and 6 weeks respectively after the treatment.

9525 P

Effects of Pinealectomy Over Several Generations.*

M. C. D'Amour and F. E. D'Amour.

From the Research Laboratories, University of Denver.

Anderson and Wolfe[1] point out that previous results of pinealectomy have been almost equally divided among those authors who report premature sexual development, frequently accompanied by increased growth, and those who obtained negative results. In their own work on 30 rats, pinealectomized at 1-3 days with adequate controls, no effect was found on rate of growth, age of puberty or weights of endocrine organs involved in growth and sexual development.

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* This investigation was supported in part by a grant from the National Research Council, Committee on Problems of Sex.

[1] Anderson, D. H., and Wolfe, A., *J. Physiol.*, 1934, **81**, 49.

It was thought that by continuing early pinealectomy over several generations, some effect might become manifest. This work was, therefore, intended as a preliminary test with a few animals with the idea that if some change did show up by the fourth or fifth generation, a more thorough and systematic study would be justified.

Four litters of rats of the same age were divided into control and test groups. The test animals of this and of later generations were all operated before reaching 48 hours of age. A diamond shaped bit of skull and dura about 2 mm. on the side and with 2 sides lying directly adjacent and parallel to the transverse sinuses was cut out over the cerebellum. The pineal, in the rat, lies directly beneath the junction of the transverse and sagittal sinuses. It is not exposed by this procedure, but by inserting the points of a tightly closing iris forceps about one mm. beneath the skull and dura at this point, the gland is almost invariably removed when the forceps are closed and withdrawn. Although the pineal is tiny, it is discrete and has a tough capsule, so that when it is removed at the first attempt there is no doubt as to the completeness of the operation. Only those animals were used in this experiment in which it was certain that the gland had been removed at the first attempt.

The babies were weaned at about 25 days. Operated rats of each generation were placed together in one large cage and their controls in another. When the females became pregnant and approached term, they were isolated.

Results. Body Weight. No significant differences in weight were observed in the first 2 generations. In the third and fourth generations, however, a distinct increase in the weight of the operated animals was noted.

TABLE I.
Weights of Pinealectomized and Control Rats of IIIrd and IVth Generations.

	Operated				Control				
	Age, mo.	No. rats	Wt.	Aver.	Age mo.	No. rats	Wt.	Aver.	Diff.
III	5	4 m.	320-365	340	5.5	6 m.	260-310	286	46
	8	4 "	340-395	363	8.5	6 "	275-315	301	62
	8	3 f.	248-295	274	8.5	3 f.	225-240	233	41
IV	1. 3.5	5 m.	280-308	291	4	5 m.	210-240	224	67
	6.5	5 "	320-350	336	7	5 "	215-270	240	96
	6.5	1 f.	274		7	2 f.	218-228	223	51
IV	2. 3.5	4 m.	276-345	304	3.5	4 m.	218-285	256	48

Because of the small size of the groups, the conclusion cannot be drawn with assurance that removal of the pineal leads to increased growth which becomes more marked in subsequent generations. The results are, however, suggestive and the problem deserves further investigations.

Somatic Maturity. Minor variations in the time of first appearance of hair and of opening of eyes and ears occurred in both operated and control groups, however the differences between the averages of the 2 groups were insignificant.

Sexual Maturity. As criterion of sexual maturity we used the ability of the males to beget and of the females to produce, young. No significant difference in this respect between the 2 groups was observed. The greatest difference in the dates of birth of the first litter in each group in the fifth generation was only 17 days. This would seem to disprove any retardation or acceleration of sexual function.

9526 P

Experimental Studies on Eggs and Miracidia of *Renifer aniarum* (Leidy, 1891) and *Dasymetra villicaeca* (Byrd, 1935).

J. HENRY WALKER. (Introduced by Ernest Carroll Faust.)
From the Parasitology Laboratory, Tulane University of Louisiana, New Orleans.

Previous investigators of trematodes of the Subfamily Reniferinæ, to which the 2 species studied by the writer belong, have found the eggs fully developed when oviposited. In the present studies 2 groups of parasite-free *Physa halei* Lea (the snail host) were inoculated October 1 and October 6, 1936, respectively with freshly oviposited eggs of *Renifer aniarum* (Leidy, 1891), which no doubt had been naturally incubated in the uteri of the trematodes while in the mouth of the snake, *Natrix cyclopion* (Duméril and Bibron), and shed cercarial larvae of this fluke 36 days later. During the 30 days previous to the dates of inoculation the mean air temperature was 28°C. in the laboratory where the snakes were kept. Subsequently 3 groups of parasite-free *P. halei* were inoculated with eggs of *R. aniarum* November 4, 11, and 20, 1936, respectively, and one group of parasite-free *P. halei* was inoculated November 20, 1936, with eggs of *Dasymetra villicaeca* Byrd, 1935. Individuals from these 4 groups were later fixed at various time intervals after the dates of inoculation and were found on careful examination to be uninfected. Daily attempts were then made to hatch freshly oviposited eggs in the digestive tract of the appropriate snail host, with negative results. During October, 1936, the mean air temperature was 22.5°C. and during November, 1936, 15.7°C.

The eggs of both species, embryonated 1-30 days at room temperature (17-20° C.) failed to mature. Experiments demonstrated that during the colder months it was necessary to incubate artificially the freshly oviposited eggs for a period of 6 days at a temperature of 25° to 30°C. to produce mature and usually viable eggs so that, after the ingestion of these eggs by the appropriate snail host, hatching would occur. Artificial incubation of eggs of both species was unnecessary after April 15, 1937.

Experiments showed that the daily average number of eggs extruded per worm was 89.21 and that the total capacity of the uterus was 2669 eggs; thus previous to oviposition the age of those eggs at the extreme distal end of the uterus must have been 29.9 days. Since 1868 eggs were found to be extruded during the first oviposition, the age of eggs at the distal end of the uterus on the second day must have been 8.97 days.

The eggs of trematodes with known life histories either (1) hatch in water and their miracidia actively penetrate the first intermediate host (usually a Gastropod) or (2) the eggs are ingested by the snail and hatching occurs in the digestive tract of the snail. *Fasciola hepatica*[1] is the classical example of the former method. A survey of the literature showed that probably 20 known species among the Families Opisthorchidæ, Heterophyidæ, Dicrocoeliidæ, Azygiidæ and Plagiorchidæ employ the latter method. All attempts to hatch the eggs of *R. aniarum* and those of *D. villicaeca* outside the digestive tract of the appropriate snail host were consistently negative. While many workers have seen living miracidia attempting penetration of the gut of a dissected snail there is only one record of a miracidium having been observed in a sectioned gut wall of an infected snail.[2]

In the present studies a large number of the appropriate snail hosts were killed and fixed at regular intervals after the ingestion of fully developed eggs of *R. aniarum* and *D. villicaeca.* A complete examination of stained serial sections has shown (1) hatched eggs in the crop-gizzard, stomach and mid-intestine of the snail; (2) free-swimming miracidia in the stomach and mid-intestine; (3) miracidia attempting to penetrate and depressing the epithelium of the stomach and intestine; (4) miracidia at various levels within the epithelium of the stomach and intestine; (5) miracidia between the epithelium and the surrounding connective tissue of the stomach and intestine; (6) the transformation of the miracidia into the mother sporocysts; and (7) all stages up to the emergence of the cercariæ.

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[1] Thomas, A. P., *Quart. J. Micros. Sc.*, 1883, **23**, 99.

[2] Ingles, L. G., *Univ. Cal. Pub. Zool.*, 1933, **19**, 143.

The consistent evidence, stage by stage, of the hatching, penetration and metamorphosis phenomena within the snail indicates for the first time the exact locations and methods by which the snail becomes infected with these *Reniferine trematodes.* Information is now available indicating the method by which those families of trematodes whose eggs do not hatch *in vitro* are able to infect appropriate snails and to proceed with development within those molluscs.

9527

Choline-Esterase Content of Normal and Denervated Submaxillary Gland of the Cat.*

F. C. MACINTOSH. (Introduced by B. P. Babkin.)
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The heightened sensitivity of the cat's submaxillary gland to sympathetic stimulation, following degenerative section of the chorda tympani, appears to be due to some spontaneous activity of the decentralized parasympathetic supply, the phenomenon thus being analogous to the well-known "augmented secretion".[1] Histological examination of the unstimulated paralytic gland, indeed, reveals changes characteristic of activity in the demilune cells,[2] which are normally controlled by the sympathetic.[3] An attractive hypothesis is that the latter elements are sensitized by acetylcholine which is liberated elsewhere in the gland through the spontaneous activity of the postganglionic neurones, and which reaches the demilune cells by diffusion. After degenerative section of the chorda tympani, however, there is no increase, but a decrease, in the acetylcholine equivalent of the submaxillary gland.[4] Since the latter term includes both combined and free acetylcholine, it might still be possible that in the paralytic gland there is a higher concentration of the *free* ester. Thus it might be thought that denervation impairs the ability of the gland to inactivate acetylcholine which has been liberated within it, by reducing the gland's supply of choline-esterase.

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* During the preparation of this paper for publication, a paper by F. Th. v. Brücke has appeared (*J. Physiol.*, 1937, **89**, 429) in which it is shown that section of the cervical sympathetic nerve in the cat greatly diminishes the choline-esterase content of the superior cervical ganglion. This case is clearly not analogous to that of the submaxillary gland, in which doubtless only a small part of the total esterase content has a special relation to the terminations of the (preganglionic) fibres which are cut.

[1] MacIntosh, F. C., and Rawlinson, H. E., *Quart. J. Exp. Physiol.*, 1935, **25**, 199.

[2] Rawlinson, H. E., *J. Anat.*, 1935, **70**, 143.

[3] Rawlinson, H. E., *Anat. Rec.*, 1933, **57**, 289.

Choline-esterase occurs in different tissues, as well as in the blood, in widely varying concentrations, the richest sources being, in general, those organs possessing a cholinergic innervation.[5] There has hitherto been no evidence to indicate whether or not the amount present in any organ is affected by section of the nerve supply to the organ. I have therefore made a series of determinations of the rate at which acetylcholine is destroyed by simple extracts of paralytic, as compared with normal, submaxillary glands.

The method used was a modification of that of Plattner and Hintner,[5] acetylcholine being assayed on the eserinized *rectus abdominis* of the frog, as described by Chang and Gaddum.[4] Aseptic section of the chorda-lingual trunk was performed on one side 12 to 20 days before the determinations. Under chloralose-urethane anesthesia the submaxillary glands were perfused with Ringer's solution at body temperature until blood-free, then rapidly dissected out of their capsules, dried superficially, and weighed. The glands were then ground with washed quartz sand in 3 volumes of M/3 phosphate buffer (pH 7.4). (The denervated gland weighed on the average 20% less than the normal, confirming Langley.[6]) At least 30 minutes' vigorous grinding was required for each gland; otherwise low values for the esterase content were obtained. The residue from the grinding, after twice washing and centrifuging, yielded very little esterase activity on repeated grinding; the great bulk of the enzyme was therefore presumably extracted. The extracts were covered and allowed to stand 3-4 hours at room temperature. Six volumes of water were added to make the final dilution of the tissue tenfold, and the mixture again centrifuged and decanted. For the assay, one cc. of the extract was added to one cc. of a 1:10,000 solution of acetylcholine chloride, and the mixture allowed to stand at room temperature for a definite time (30-300 seconds). One cc. of the mixture was then added to the bath (volume 100 cc.) containing the eserinized rectus, and the resulting contraction compared with that produced by a known dose of acetylcholine. Control experiments showed that the presence of gland materials did not affect the reaction of the rectus to acetylcholine simultaneously or subsequently applied to it. The enzymatic destruction of the acetylcholine was quickly halted by the eserine present in the bath: precipitation with trichloracetic acid, as in the method of Plattner and Hintner, was therefore unnecessary. At least 3 determinations were made with each extract.

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[4] Chang, H. C., and Gaddum, J. H., *J. Physiol.*, 1933, **79**, 255.

[5] Plattner, F., and Hintner, H., *Pflügers Arch.*, 1930, **229**, 19.

[6] Langley, J. N., *J. Physiol.*, 1885, **6**, 71.

From the values obtained a curve was plotted, from which was read the time required for 50% destruction of the added acetylcholine. The results obtained are given in Table I, the values for the gland from the same animal being grouped in pairs.

TABLE I.

Gland	Time required for 50% destruction of added acetylcholine (minutes)
Normal (left)	1¾
” (right)	1¾
” (right)	1¼
” (left)	2½
Paralytic (right)	2
Normal (left)	1¾
Paralytic (right)	2¼
Normal (Plattner and Hintner)	5

The acetylcholine-inactivating power of the cat's submaxillary gland is thus somewhat higher than that determined for the dog's submaxillary gland by Plattner and Hintner, the dilutions of the tissue being the same in both cases.

Since the catalytic destruction of acetylcholine is a monomolecular reaction, the assay can be checked by ascertaining the rate of inactivation at different times after the addition of the esterase solution. This, of course, is proportional to the concentration of the unhydrolyzed ester, *i. e.*,

$$k = \frac{1}{t}\log\left(\frac{a}{a-x}\right)$$

where k is constant and a and a-x are the concentrations of acetylcholine after 0 and t seconds respectively. Table II illustrates the results when this calculation is made. Considering the rather large error of the biological assay, the values for k determined for the same extract show tolerably good agreement.

TABLE II.

Gland	t	$a-x\,(a=100)$	$k=\frac{1}{t}\log\left(\frac{a}{a-x}\right)$
Left (normal)	60	75	.00208
	120	57.5	.00200
	240	30	.00218
Right (paralytic)	50	75	.00250
	60	70	.00258
	75	67.5	.00228
	150	42.5	.00248

Solutions of acetylcholine subjected to prolonged treatment with gland extracts lost all perceptible activity.

Statistical analysis of a much larger series of determinations would be necessary to determine whether denervation of the gland has any definite influence on its esterase content. Nevertheless it is clear that this is of the same order of magnitude in the normal and denervated gland, and that no difference exists which can adequately explain the tremendously increased sensitivity of the paralytic gland to sympathetic stimulation. It is, however, still possible that degenerative section of the chorda stimulates the production of free acetylcholine within the gland. The *chorda tympani* fibres cut were, of course, preganglionic, and it is possible that degeneration of the postganglionic fibres might significantly affect the esterase content of the gland: unfortunately these are inaccessible to section.

The production of enzymes by other glandular tissues continues after section of the nerves which normally control these enzymes. A familiar example is provided by the Heidenhain pouch of the stomach, which has no extrinsic vagal innervation but continues indefinitely to elaborate pepsin. Nothing is known of the conditions under which choline-esterase is formed in the body. Its high concentration in the blood would suggest, indeed, that the intervention of the nervous system is unnecessary.

9528 P

Influence of Potassium on Blood Sugar and Tissue Glycogen.

H. Silvette* and S. W. Britton. (Introduced by H. E. Jordan.)

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It has been suggested that most of the disturbances observed after adrenal removal are secondary to a primary defect in potassium metabolism.[1] For the specific case in which we are interested, this is held to mean that the low blood sugar and tissue glycogen of adrenalectomized animals result from a primary rise in serum potassium. Some of the experiments we have been carrying out recently bear on this hypothesis, but lend it no credence.

Normal white rats weighing between 80 and 100 gm. were fasted for 18 hours, given intraperitoneal injections of K-free or K-containing solutions, and then serially sacrificed. When the injected solution consisted of normal sodium chloride and 5% glucose solution (10 cc. per 100 gm. rat), the blood sugar and liver and muscle glycogen curves over 3.75 hours followed the course shown in Table I. Both muscle and (especially) liver glycogen were deposited in large amounts, while the blood sugar level remained relatively stationary. But when the injected fluid contained, in addition to so-

TABLE I.
Effect of Potassium Injections on Blood Sugar and Glycogen.

Solution containing 0.9% NaCl, 5% glucose and	Animals killed after injection, hrs.	Blood sugar, mg. %	Liver glycogen, %	Muscle glycogen, %
No K	0.75	123	3.40	0.44
	1.5	116	3.29	0.52
	2.25	132	3.38	0.59
	3.	110	2.39	0.47
	3.75	117	3.64	0.44
0.2% K	0.75	122	4.04	0.50
	1.5	117	2.65	0.46
	2.25	131	1.52	0.43
	3.	105	2.37	0.40
	3.75	98	2.10	0.41
0.4% K	0.75	144	0.37	0.35
	1.5	222	0.59	0.38
	2.25	141	0.88	0.34
	3.	146	0.83	0.34
	3.75	102	0.74	0.39

Each figure is the average of 3 experiments.

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* E. R. Squibb and Sons Scientific Fellow in Physiology.

[1] Truszkowski, R., and Zwemer, R. L., *Biochem. J.*, 1936, **30**, 1345.

dium chloride and glucose, 0.4% potassium (in the form of potassium acetate), the formation of both muscle and liver glycogen was completely suppressed, while the blood sugar level quickly rose over 100%. The administration of this amount of potassium (40 mg. per 100 gm. body weight) produced no visible toxic effects, and the rats when sacrificed were in as good condition as the controls. When half the quantity of potassium was injected (*i. e.,* 20 mg. per 100 gm.), the blood sugar and tissue glycogen values observed did not differ significantly from the control levels.

Other experiments performed on animals under somewhat different conditions (fully-fed; urea-injected, etc.) confirmed the conclusion that the administration of sub-toxic doses of potassium leads to a great increase in blood sugar and a diminution of the tissue glycogen levels; in other words, to glycogenolysis and hyperglycemia. It is pertinent in this connection to recall that MacQuarrie, *et al.,*[2] observed the administration of potassium salts to decrease the glucose tolerance of diabetic children. To contend, therefore, that the increased potassium level observed in adrenal insufficiency is the cause of the lowered blood-sugar levels characteristic of that condition is contrary to the above experimental facts. It is very significant that, in spite of the heightened serum potassium concentration, the blood sugar levels after adrenalectomy are found to be subnormal.

9529

Effect of Prolonged Insulin Therapy on Glucose Tolerance in Schizophrenic Patients.

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The treatment of schizophrenic patients by the insulin hypoglycemia method of Sakel[1] has been carried on in this institution for the past year by Cameron and Hoskins.[2] In connection with this treatment a systematic investigation of the changes produced in various physiologic functions has been undertaken to determine, if possible, the mechanism by which amelioration of the schizophrenic process is brought about. Among these the glucose tolerance was determined before treatment was initiated and also after insulin had been given for a considerable period.

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[2] MacQuarrie, I., Thompson, W. H., and Anderson, J. A., *J. Nutrition,* 1936, **11**, 77.

[1] Sakel, M., *Neue Behandlungsmethode der Schizophrenie,* Moritz Perles, Wien und Leipzig, 1935.

[2] Cameron, D. E., and Hoskins, R. G., *J. A. M. A.,* in press.

In all cases the blood sugar was estimated by the method of Folin and Wu after giving 100 gm. of glucose by mouth while the patient was in a fasting state. Blood samples were taken before the sugar was given and at one half hour, one hour, and 2 hours after its ingestion.

The results of the analyses are given in Table I for the 9 patients studied. The treatment was carried out approximately 6 days a week for the period indicated in the table before the second tolerance test was made.

TABLE I.
Blood Sugar Values after 100 gm. of Glucose, mg./100 cc.

						Treatment	
Case	Date	Fasting	½ hr.	1 hr.	2 hr.	From	To
M.L.	1/22/37	110	146	152	123	1/26/37	5/19/37
♀	5/20/37	114	234	214	172		
E.B.	11/5/36	87	145	128	120	11/9/36	5/19/37
♂	5/20/37	96	161	140	135		
T.S.	9/22/36	70	106	136	91	11/2/36	12/23/36
♂	4/8/37	112	168	176	120	2/15/37	3/22/37
R.W.							
♂	2/4/37	108	204	260	158	6/29/36	2/3/37
M.B.	3/25/37	75	111	70	76	3/29/37	5/19/37
♀	5/20/37	93	102	238	178		
A.S.	11/25/36	89	109	82	86	2/1/37	2/3/37
♂	2/4/37	103	206	154	not collected		
S.Mc.	12/3/36	93	118	72	60	12/8/36	2/1/37
♂	2/2/37	89	150	145	102		
A.C.	1/14/37	90	150	116	77	1/18/37	4/13/37
♂	4/22/37	96	180	120	69		
J.C.	12/3/36	92	120	93	84	12/7/36	2/1/37
♂	2/2/37	113	147	145	123		

In all the cases but one there was a definite decrease in the glucose tolerance of the patients following the repeated injection of insulin. In this case (A.C.) the maximum value for the blood sugar obtained after the ingestion of the same amount of sugar was 30 mg. % higher in the second test than in the first, but the

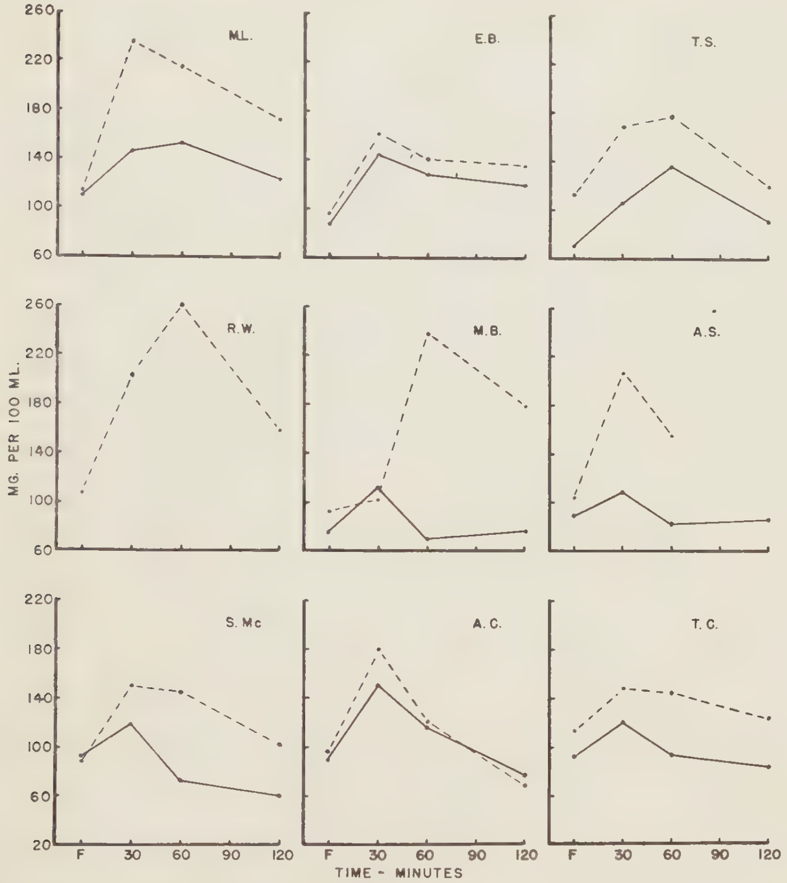

Fig. 1.

Blood sugar curves after the ingestion of 100 gm. of glucose. Solid line—Curve obtained before insulin therapy. Dotted line—Curve obtained after therapy.

height was not sustained as in the other cases. These changes are shown in Fig. 1. The curve for the patient (R.W.), who had been under treatment for the longest period showed the greatest elevation in blood sugar. Unfortunately no preliminary test was made in this case before treatment was instituted. However, no evidence that he was diabetic was found in routine examinations.

It is also to be noted that the initial fasting levels for the blood sugar were significantly higher in the majority of the cases at the second test over the values obtained in the first test.

The explanation for this change in the physiological response to sugar has not been determined. Two possibilities suggest themselves, first, that a stimulation of the pituitary has taken place so that the insulin antagonistic factor is produced in greater amounts, and second, that a sensitization and stimulation of the adrenal-sympathetic system has occurred.

In view of the second possibility it is interesting to note that previous investigations from our laboratories have indicated that adrenal insufficiency may be a factor in the schizophrenic reaction, hence a change in the adrenal-sympathetic mechanism might correlate with the improved clinical status of the patients under insulin treatment. These patients have been noted by Hoskins and Jellinek[3] to be characterized by a sluggish sympathetic reactivity. The correlation between systolic and diastolic blood pressures is significantly higher in the schizophrenic than in the normal subject. This would indicate that in the schizophrenic patient the cardiovascular system operates more as a mechanical hydrostatic system and is less subject to homeostatic influences than that of the normal subjects. After injecting adrenalin there was a disappearance of the correlation, the patients acting more like the normal group. Furthermore, most schizophrenic patients react with an increase in blood pressure to the oral administration of glycerine extract of the adrenal cortex, whereas relatively few normal subjects are affected by this medication.[4]

The theory is offered that repeated injections of large amounts of insulin increase the antagonistic activity of the adrenal glands. When glucose is administered by mouth, the increased concentration in the blood provokes the liberation of insulin and this in turn elicits the adrenal response with the liberation of adrenalin. The adrenalin prevents the formation of glycogen in the liver and the blood sugar remains elevated because of a slowing down of its removal. This is in accord with the proposal of Soskin, *et al.*,[5] that the liver is the main homeostatic agent preventing the increase of blood sugar after the ingestion of glucose.

Summary. In 7 schizophrenic patients undergoing insulin therapy there was a significant decrease in sugar tolerance, the blood sugar levels after glucose administration attaining higher values and remaining elevated for longer periods than before treatment. In a ninth patient in whom a preliminary determination was lacking, the tolerance was notably low, also. This change may have been due to the stimulation and sensitization of the adrenal mechanism.

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[3] Hoskins, R. G., and Jellinek, E. M., *Proc. Assn. Research in Nervous and Mental Diseases*, 1933, **14**, 211.

[4] Hoskins, R. G., and Freeman, H., *Endocrinology*, 1933, **17**, 29; Freeman, H., Linder, F. E., and Hoskins, R. G., *Endocrinology*, 1933, **17**, 677; Freeman, H., and Hoskins, R. G., *Endocrinology*, 1934, **18**, 516.

[5] Soskin, S., Mirsky, I. A., Zimmerman, L. M., and Heller, R. C., *Am. J. Physiol.*, 1936, **114**, 648.

9530 P

Hyperplasia of Uterine Muscle, as Studied by the Colchicine Method.

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Distention of the uterus is undoubtedly a factor in stimulating its growth during pregnancy.[1, 2, 3] The scarcity of mitotic figures in muscle during the enlargement of the pregnant uterus has led to the usual textbook statement that increase in size is due to hypertrophy rather than to hyperplasia of the muscle fibers. It is usually difficult to find dividing cells in uterine muscle even when the endometrium is undergoing marked hyperplasia in response to stimulation by ovarian follicular hormone.

The use of the drug, colchicine, which arrests mitoses in metaphase and thus accentuates hyperplasia[4, 5, 6] has made the search for cell division much more effective.

In a first series of short experiments (48 hours) with theelin and colchicine in adult ovariectomized mice, mitotic figures were abundant in the surface epithelium and glands of the uterus, but very scarce in the uterine muscle.[7, 8]

In later experiments, where injections of theelin, prephysin and pregnant mares' serum were made for longer periods in conjunction with colchicine (0.1 to 0.3 mg.) into puberal or young adult mice

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* Fellow of the John Simon Guggenheim Memorial Foundation.

[1] van Dyke, H. B., and Gustavson, R. G., *J. Pharm. and Exp. Therap.*, 1929, **37**, 379.

[2] Markee, J. E., and Hinsey, J. C., *Anat. Rec.*, 1935, **61**, 311.

[3] Markee, J. E., Wells, W. M., and Hinsey, J. C., *Anat. Rec.*, 1936, **64**, 221; Reynolds, S. R. M., *Am. J. Obstet. and Gynec.*, 1937, **33**, 968.

[4] Dustin, A. P., *Jour. des Sci. Med. de Lille*, 1934, No. 49, 561.

[5] Lits, F., *C. R. Seance de Biol. Belge*, 1934, **115**, 1421.

[6] Ludford, R. J., *Arch. f. Exp. Zellforsch.*, 1936, **18**, 411.

[7] Allen, E., Smith, G. M., and Gardner, W. U., *Anat. Rec.*, 1936, **67**, (Suppl. 1), 49.

[8] Allen, E., Smith, G. M., Gardner, W. U., *Am. J. Anat.*, in press.

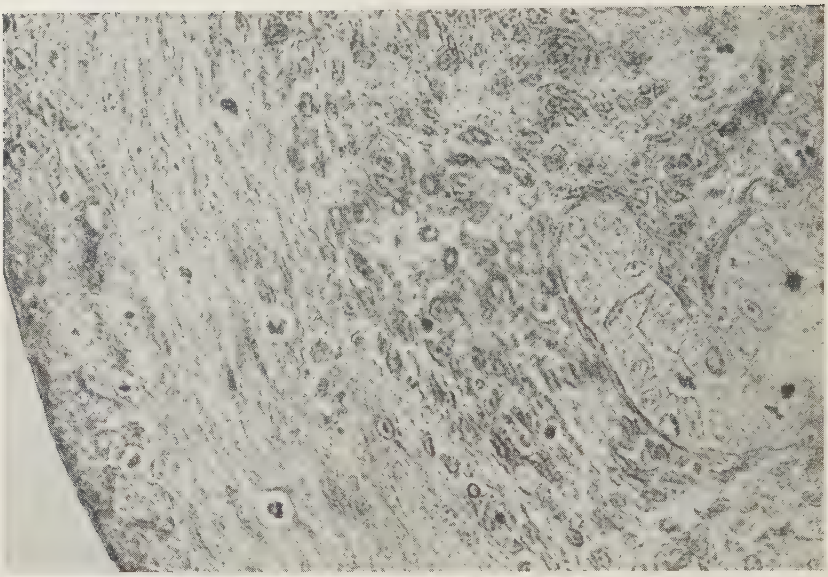

FIG. 1.
Frequent mitoses in circular and longitudinal muscle of the uterus (at left) of the mouse injected with prephysin and colchicine. Prephysin stimulates development of ovarian follicles which secrete folliculin (estrone) causing growth of the uterus. Colchicine arrests mitosis in metaphase, thus accentuating the mitotic index.

and rats,[9] mitoses were frequent in both circular and longitudinal muscle (Fig. 1). It was noted that the maximum incidence of mitosis occurred when there was distention of the uterus with secretion as occurs in both mouse and rat at estrus. This brought up the old question, "How much of this hyperplasia is due to hormonal stimulation and how much to the stimulus of distention?" The endometrium, both epithelial structures and stroma, contained frequent mitoses and the vagina, tubes, and mammary glands were also very hyperplastic. Since most of these tissues are not subjected to distention the endocrine factor must be partly responsible.

At this point Dr. Reynolds visited our laboratory and we discussed the above results and his recent findings that distention of the rabbit's uterus with paraffin pellets, even in ovariectomized animals, causes an increase in size as measured with the planimeter.[10] This led to the following preliminary experiment, with the colchicine method, to find out how much of the increased size of the distended uterus is due to hyperplasia.

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[9] Allen, E., Smith, G. M., and Gardner, W. U., *Anat. Rec.*, 1937, **67** (Suppl. 3), 3.

[10] Reynolds, S. R. M., and Kaminester, S., *Am. J. Physiol.*, 1936, **116**, 510.

Four rabbits were ovariectomized on March 11th. On March 17th, several moulded pellets of 2 sizes were inserted in one uterine horn of each animal as described previously.[10] The animals were killed at 27, 50, 73, and 98 hours after insertion of the pellets. Approximately 18 and 9 hours before sacrificing, 2 injections of 0.3 mg. each of colchicine were made to hold in metaphase all cells dividing during the last 18 hours of life. This method accumulates evidence of hyperplasia and makes mitotic indices much more convincing.

A preliminary study of sections shows definite hyperplasia was at a maximum at 73 hours in the uterine segments distended with the larger sized pellets. There were a few mitoses in the 98-hour stage. There were very few mitoses in the endometrium—occasional ones being located in the glands. The endometrium was infiltrated with leucocytes. This and some edema might account for most of the increase in size of this part of the uterus but this point requires further study. At the same time a study of the effect of trauma incident to vigorous smearing of ovariectomized rats has shown (by means of the colchicine technique) that this also stimulates partial estrous growth.[11]

This work must be extended and the problem studied much more thoroughly. The necessity of postponement of further attack made this preliminary report seem worthwhile.

Summary and Conclusions. (1) The muscles of the uterus of the ovariectomized mouse and rat undergo hyperplasia in response to ovarian follicular hormone (theelin) and a similar response is obtained in normal animals injected with prephysin and pregnant mares' serum. (2) The mitotic index, accentuated by arrest of mitosis in metaphase with colchicine, makes this new evidence especially convincing. (3) The highest index is obtained after 2 or more days of hormone action when the uterus is distended by secretion, as occurs at estrus in the mouse and rat. (4) The uterus of the ovariectomized rabbit, distended with paraffin pellets, but not treated with sex hormones, also shows hyperplasia at the sites of distention. As many as 50 mitoses may be counted in the muscle in one cross section of the uterus after 73 hours of distention. The endometrium in the same section contains few mitoses, is infiltrated with leucocytes and shows other signs of castrate atrophy. (5) Apparently both (*a*) endocrine stimulation and (*b*) distention are factors in hyperplasia of uterine muscles. The second factor alone induces hyperplasia in muscle tissue in the ovariectomized rabbit.

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[11] Rogers, P. V., and Allen, E., *Endocrinology,* in press.

9531

Adrenalectomy in Experimental Hypertension from Kaolin.

W. A. Jeffers, M. A. Lindauer and F. D. W. Lukens. (Introduced by J. H. Austin.)

From the Robinette Foundation, the George S. Cox Medical Research Institute, and the Pepper Laboratory of the Hospital of the University of Pennsylvania.

A possible rôle of the adrenal glands in the maintenance of arterial hypertension has been the subject of recent clinical and animal investigation. Goldblatt[1] has suggested that the adrenal cortex is concerned in the maintenance of blood pressure in dogs made hypertensive by constriction of the renal artery.

In our experiments dogs were made hypertensive by intracisternal kaolin injection by the method of Dixon and Heller.[2] There is evidence that this type of arterial hypertension is not affected by denervation of the adrenal glands.[3] Because denervation is presumed to affect only the medullary secretion, it was felt that complete adrenalectomy might yield evidence concerning the importance of the adrenal cortex to this type of hypertension.

Blood pressure measurements were made daily, using the method of Allen, as modified by Collins.[4] After a control period of 7 to 14 days the right adrenal gland was removed. About 10 days later kaolin was injected into the cisterna magna. After 7 to 10 days of arterial hypertension (160-300 mm. of Hg systolic) left adrenalectomy was performed. The dogs were then maintained free from cortical insufficiency by the use of an adrenalin-free cortical extract (Upjohn) (1-3 cc. per day), and salt (sodium chloride, 4 to 6 gm. per day, or a mixture of 6 gm. sodium chloride and 5 gm. sodium citrate).

Four hypertensive animals survived the removal of both adrenal glands. One of them showed a gradual fall in blood pressure to the range of 90-140 mm. of Hg following the second adrenalectomy, without any signs of cortical insufficiency. The other 3 exhibited persistent arterial hypertension (160-250 mm. of Hg) after the second adrenalectomy, as long as they were well maintained by the use of cortical extract and salt. We attempted to keep 2 of the hypertensive animals using only salt. These efforts were unsuccessful.

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[1] Goldblatt, H., *Ann. Int. Med.*, 1937, **11**, 69.

[2] Dixon, W. E., and Heller, H., *Arch. f. exp. Path. u. Pharmakol.*, 1932, **166**, 265.

[3] Hogler, F., Uberrack, K., Zell, F., and Falta, W., *Klin. Wchnschr.*, 1934, **13**, 255.

[4] Collins, D. A., *Am. J. Physiol.*, 1936, **116**, 616.

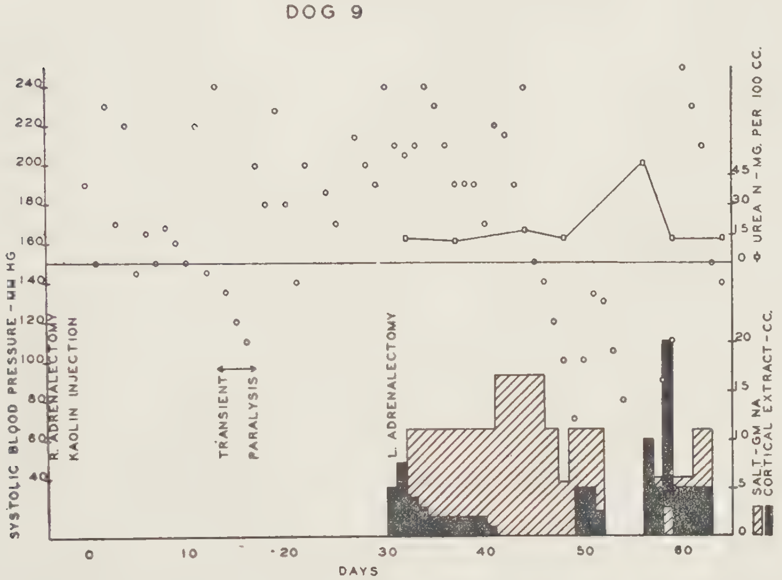

FIG. 1.

Effect of adrenalectomy upon hypertension which has been induced by an intracisternal injection of kaolin. Circles show systolic blood pressure.

Signs of cortical insufficiency soon appeared and the blood pressure fell to the range of 80-100 mm. of Hg. Upon resumption of adequate doses of cortical extract there was evidence of recovery, and a coincident return of blood pressure to the previous hypertensive range.

The results confirm the contention of Hogler, Uberrack, Zell, and Falta[3] that the adrenal medulla is not necessary for this type of hypertension.

It appears that the adrenal cortex, or an extract of it, is necessary for the maintenance of both normal and hypertensive blood pressure levels. It may be pointed out, however, that whereas three times the usual maintenance dose of cortical extract fails to replace the action of the normal adrenal cortex in an adrenalectomized depancreatized animal,[5] no increase above the maintenance dose is needed to support this type of hypertension. The significance of the amount of cortical hormone required under varying conditions is not yet understood.

Summary. Three out of 4 dogs made hypertensive by intracisternal injections of kaolin showed a persistent elevation of arterial blood pressure following bilateral adrenalectomy, as long as they were adequately maintained by the use of salt and of a non-pressor extract of the adrenal cortex. The fall in blood pressure of the fourth animal was attributed to the spontaneous remission of the experimental hypertension.

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[5] Long, C. N. H., and Lukens, F. D. W., *J. Exp. Med.*, 1936, **63**, 465.

9532

Influence of Ergotamine Tartrate Upon Peripheral Blood-Flow in Subjects with Liver Disease.

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It has been established in animals[1-5] and in man[6] that ergotamine tartrate in therapeutic doses generally constricts peripheral arteries with a consequent decrease in blood flow. The recent reports of the occurrence of peripheral gangrene following the use of this drug[7, 8] for the relief of pruritus associated with jaundice[9, 10] raises the question as to whether the presence of liver insufficiency might not influence quantitatively this response of the blood vessels. Accordingly, the effect of a single subcutaneous injection of ergotamine tartrate (0.5 mg.) upon the rate of blood-flow was determined in a series of patients with liver disease and compared with that in a control group.

The principles and details of the method of determining blood-flow outlined by Brodie,[11] and Hewlett and Van Zweluwenburg[12] were adhered to. The subjects were allowed a light repast at least 2 hours before the test. They rested comfortably in the supine position, covered with a light blanket in a room at a constant temperature, usually about 24°C. The hand was placed in a plethysmograph possessing the improved modifications of Freeman[13] and the machine was filled with water which was maintained at a temperature of 32°C. All air was excluded. A rubber cuff about 1.5 inches in width encircled the wrist approximately 2 inches above the rubber diaphragm of the plethysmograph. In order to obtain blood-flows, the cuff was suddenly inflated at a pressure of 70 mm. of mercury from a pressure bottle so as to obstruct the venous outflow from the hand; the increase in hand volume at the end of the first second after the application of the pressure was accepted as a measurement of unimpeded arterial flow into the limb. This increase in volume was recorded on a kymograph by means of a Brodie's bellows and a lever pen. After a control period of 30 minutes, during which time determinations of blood-flows were obtained every 3 minutes, 0.5 mg. of ergotamine tartrate (Sandoz) was injected subcutaneously. For 40 to 60 minutes thereafter, readings were made at intervals of 3 to 5 minutes. In no instance did the drug cause emesis or nausea.

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* Work done under the Tenure of the Richard and Ella Hunt Sutro Fellowship in Cardiovascular Research.

† Mr. J. Marrus rendered expert technical assistance.

[1] Dale, H. H., *J. Physiol.*, 1906, **34**, 163.

[2] Lewis, T., and Gelfand, B., *Clin. Sc.*, 1935, **2**, 43.

[3] Herrick, J. F., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 271.

[4] Pool, J. L., and Nason, G. I., *Arch. Neurol. and Psychiat.*, 1935, **35**, 276.

[5] Rubin, M. I., and Rapoport, M., *Arch. Int. Med.*, 1937, **59**, 714.

[6] Graham, J. R., and Wolff, H. G., Reported before The Am. Soc. for Clin. Invest., Atlantic City, May 3, 1937.

[7] Yater, W. M., and Cahill, J. A., *J. A. M. A.*, 1936, **106**, 1625.

[8] Gould, S. E., Price, A. E., and Ginsberg, H. I., *J. A. M. A.*, 1936, **106**, 1631.

[9] Lichtman, S. S., *J. A. M. A.*, 1931, **97**, 1463.

[10] Snell, A. M., and Keyes, H. C., *M. Clin. N. Am.*, 1933, **16**, 1455.

[11] Brodie, J. G., Seventh International Physiological Congress, Aug., 1907.

[12] Hewlett, A. W., and Van Zweluwenburg, J. G., *Arch. Int. Med.*, 1909, **3**, 254; *Heart*, 1909, **1**, 87.

The system was calibrated by injecting air in one cubic centimeter amounts with the aid of a syringe and the results were expressed as cubic centimeters of blood-flow per minute per 100 cc. of hand volume. The latter was determined by subtracting the figure representing the amount of water added to the plethysmograph with the hand *in situ* from that representing the actual capacity of the machine. Various details of the procedure and apparatus are being standardized by one of us (D.I.A.)[14] to assure reliable absolute blood-flow values. However, the method used here was considered adequate for the purpose of comparing relative changes in blood-flow in the same subject before and after a stated procedure.

The blood-flow in the hands of 3 normals and 3 patients without established liver abnormality was compared with that in 6 patients with liver disease or jaundice. Five of the 6 patients in the control group showed a decreased blood flow after the injection of ergotamine, 13 to 61% lower than their initial flows. In most instances this decrease was evident in the first 10 minutes after the drug was given, becoming more pronounced later in the experiment. Observations were not continued for longer than 50 minutes after the injection as the subjects at that time usually exhibited fatigue and became restless. In one instance, Case No. 6, there was a slight rise

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[13] Freeman, N. E., *Am. J. Physiol.*, 1935, **113**, 384.

[14] Abramson, D. I., unpublished observations.

TABLE I.
Hand Blood-Flow in Normals and in Subjects without Liver Disease Following the Subcutaneous Injection of 0.5 mg. of Ergotamine Tartrate.

Case No.	Age	Sex	Diagnosis	Av. Flow Before cc./min./100 cc. Hand Vol.	Av. Flow After Injection 10 minute periods					Maximal Change* in Rate of Flow, %	Blood Pressure		Heart Rate	
					10	20	30	40	50		Before	After	Before	After
1. P.D.	22	Male	Normal	6.7	7.2	4.4	3.8	2.6	—	—61	$\frac{94}{60}$	$\frac{90}{70}$	88-112	88-96
2. I.S.	46	”	”	12.5	9.4	7.0	5.8	5.8	5.8	—54	$\frac{135}{90}$	$\frac{135}{110}$	80	72-80
3. J.M.	31	”	Conversion Hysteria	7.3	6.0	4.6	4.6	4.5	—	—39	$\frac{130}{70}$	$\frac{140}{92}$	80	70-80
4. K.B.	24	Female	Migraine	13.5	11.3	11.8	10.0	9.3	—	—14	$\frac{105}{58}$	$\frac{118}{90}$	72-84	64-80
5. D.H.	47	Male	Peptic Ulcer	7.0	7.7	7.0	7.0	6.1	—	—13	$\frac{130}{80}$	$\frac{120}{80}$	80-96	60.84
6. L.S.	36	”	” ”	6.9	8.3	8.1	8.0	7.4	—	+7	105	112	80	64-84

*The maximum increase or decrease following the injection of ergotamine was calculated by comparing the resting blood-flow with the figure obtained at the end of the period of observation which in all cases manifested the maximum change.

TABLE II.
Hand Blood-Flow in Patients with Liver Disease.

Case No.	Age	Sex	Diagnosis	Av. Flow Before cc./min./100 cc. Hand Vol.	Av. Flow After Injection 10 minute periods					Maximal Change in Rate of Flow, %	Blood Pressure		Heart Rate	
					10	20	30	40	50		Before	After	Before	After
7. J.Ba.	32	Male	Subacute Liver Atrophy	15.7	15.4	13.1	13.2	12.6	—	—20	$\frac{100}{70}$	$\frac{115}{80}$	72	64-72
8. J.Br.	29	,,	Salvarsan Hepatitis	16.0	19.1	11.8	15.5	8.0	—	—50	$\frac{110}{65}$	$\frac{120}{90}$	72	48-68
9. H.B.	44	,,	Cirrhosis Hematemesis	35.8	38.5	33.0	34.8	34.8	35.8	0	$\frac{110}{65}$	$\frac{130}{70}$	84	60-76
10. M.R.	40	,,	Carcinoma Pancreas Jaundice	12.7	8.9	11.7	10.2	9.3	8.0	—37	$\frac{120}{90}$	$\frac{130}{90}$	78	60-74
11. A.B.	60	,,	Carcinoma Pancreas Jaundice	23.4	22.5	20.7	19.9	18.4	—	—22	$\frac{135}{75}$	$\frac{140}{90}$	76	74-80
12. E.A.	53	Female	Cholangitic Cirrhosis	4.0	4.6	5.7	6.0	7.2	8.6	+215	$\frac{129}{80}$	$\frac{130}{80}$	72	64-72

in blood-flow of 7% above the initial value. The initial blood-flows of the group averaged 6.7 to 13.5 cc. per minute per 100 cc. of hand volume.

In the 6 patients with liver disease, 4 gave evidence of decreased blood-flow. In the latter, after the injection of ergotamine, the original values fell 20 to 50%. In one instance, case No. 9, no change in blood-flow was recorded while in another, case No. 12, the blood-flow increased over 200%. This was still evident 50 minutes after injection. The initial blood-flows in this group ranged between 4 and 35.8 cc. per minute per 100 cc. of hand volume.

In 2 of the control and in 3 of the liver cases there was a definite rise in blood pressure of 10 to 20 mm. of mercury, while in all but one case there was a slight but definite decrease in heart rate (Tables I and II). In no instance did these changes persist during the entire period of altered peripheral blood flow.

The vasoconstrictor action of ergotamine tartrate is here confirmed in human beings by plethysmographic studies. In 2 instances, however, a vasodilator action was noted, which is in accord with previous observations.[4, 6, 15] The rise in systemic blood pressure and the decrease in heart rate following the injection were too slight and not constant or persistent enough to account for the prolonged changes in blood-flow. Further, the same degree of change was observed in those patients manifesting an increase in flow as in those with a decrease. Nevertheless, since cardiac outputs were not measured, an effect of the drug on the heart cannot be ruled out entirely.

There was no significant difference in the vascular response of subjects with liver disease from that of normals to the subcutaneous injection of ergotamine tartrate. Approximately the same degree of vasoconstriction, as indicated by the percentage decrease in peripheral blood-flow, results in both groups. This conclusion, of course, can at present only be applied to the effect of single therapeutic doses. However, it is probable that where conservative doses of ergotamine tartrate produce cardiovascular complications, an explanation may be found in previously existing disease of the vessels, unusual degrees of vasoconstriction, or unusual concentration of the blood elements, as in polycythemia.[16]

Summary. Single doses of ergotamine tartrate (0.5 mg.), injected subcutaneously, produce definite vasoconstriction as indicated by a decrease in peripheral blood-flow. Less commonly, vasodilata-

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[15] Bouckaert, J. J., and Moraes, A., *Compt. rend. Soc. de Biol.*, 1932, **110**, 993.

[16] Lichtman, S. S., Letter, *J. A. M. A.*, 1936, **107**, 148.

tion is produced, with increased blood-flow. No significant difference in blood-flow change after ergotamine was noted between normals and subjects with liver disease.

9533 P

Effect of Some Short Acting Barbiturates on the Patency of the Glottis.

C. L. BURSTEIN. (Introduced by Co Tui.)

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Experiments were conducted on normal cats with the following barbituric acid derivatives: sodium N-methyl-cyclohexanyl barbiturate (evipal), sodium iso-amyl ethyl barbiturate (amytal), sodium iso-amyl ethyl thio-barbiturate (sodium thio-ethamyl*), sodium N-ethyl-l-methyl butyl barbiturate (nembutal), and sodium N-ethyl-l-methyl butyl thio-barbiturate (pentothal). In each case a 2% aqueous solution, freshly prepared, was injected intravenously at a constant rate of a milligram per second. The quantities injected ranged between the minimum anesthetic dose and the minimum lethal dose.

It was noticed that most of the animals would cough, sneeze, or hiccup considerably during the course of anesthesia and that these animals would quickly develop cyanosis and dyspnea regardless of whether a large or small dose had been administered. Inspection of the glottis in these cases showed spastic adduction of the vocal cords. In the cases where there was no spontaneous coughing, inspection of the glottis showed hyper-active adducted vocal cords and lifting the epiglottis would elicit complete spastic closure of the glottis; cyanosis then developed rapidly and unless tracheal intubation was

TABLE I.

Barbiturate	No. of animals	Spontaneous laryngospasm	Hyperactive laryngeal reflex, followed by laryngospasm
Sodium thio-ethamyl	37	36	1
Evipal	10	7	3
Amytal	10	4	5
Nembutal	10	6	4
Pentothal	11	8	3

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* Parke, Davis & Co.

performed cardiac arrest would ensue. These results are illustrated in Table I.

Since artificial stimulation of the superior laryngeal nerve[1] causes reflex closure of the glottis by contraction of the adductor muscles, it is possible that the symptoms here observed are of parasympathetic origin. In order to test the validity of this assumption experiments were performed to study the modification of this symptom-complex by drugs affecting the sympathetic and parasympathetic divisions of the autonomic nervous system and by section of the vagi. The results may be summarized thus:

Atropin sulphate (3 to 5 mg. per kilo intravenously) always caused immediate relaxation of the vocal cords, a return of normal respiration, disappearance of cyanosis, a shortening of anesthesia time, and an increase in tolerance to the M.L.D.

Ephedrin sulphate, in therapeutic doses (20 mg. per kilo intravenously) caused only partial improvement characterized by relief of dyspnea and slight relief of laryngospasm but with persistence of hyper-active laryngeal reflex. Increasing the dose caused complete relaxation of the vocal cords but was usually followed by ventricular fibrillation.

Adrenalin hydrochloride (0.15 mg. per kilo intravenously) caused immediate improvement but the untoward symptoms reappeared after 10 to 15 minutes.

Finally, bilateral cervical vagotomy invariably relieved the laryngospasm.

From the above data it is concluded that the closure of the glottis following the intravenous administration of these short-acting barbiturates in cats is due to stimulation of the parasympathetic division of the autonomic nervous system.

9534

Use of Cerebro-Spinal Fluid and Synthetic Salt Solutions in Studies of Tissue Metabolism.

BENJAMIN ALEXANDER AND A. BAIRD HASTINGS.

From the Department of Biological Chemistry, Harvard Medical School, Boston.

The desirability of having available a physiologically normal fluid medium in which tissue slices may be immersed for the study of their metabolism is widely recognized. The use of serum as such a

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[1] Howell, W. H., *Textbook of Physiology*, ninth edition, p. 697.

TABLE I.
Composition of the Synthetic Medium.

Salt	Concentration of Stock Solution $\frac{mM}{L}$	Amt. Required per 100 cc. cc.	Final Concentration $\frac{mM}{L}$
NaCl	154	77.5	119
KCl	154	2.6	4
$CaCl_2$	104	1.15	1.2
$MgCl_2$	104	1.44	1.5
Phosphate Buffer (7.4)	67	0.75	0.5*
Phenol Red	0.1%	0.75	0.00075%
After mixing, and saturating the above solutions with 5.5% CO_2:94.5%O_2, $NaHCO_3$ solution was added as follows:			
$NaHCO_3$	154	15.8	24.3
Glucose	—	—	0.2%
pH			7.4

*The amount of phosphate added varied from 0.49 to 0.63 mM/L depending upon the amount present in the spinal fluid.

medium, though possible, presents technical complexities which preclude its wide application. It was suggested by Davis and Hastings[1] that cerebro-spinal fluid, being a protein-free biological fluid, had the advantages but not the disadvantages of serum. A comparison of the metabolism of liver in cerebro-spinal fluid and their standard synthetic solution indicated that oxygen consumption determinations made in the spinal fluid were greater than those made in their synthetic solution. These authors did not attempt to prepare a synthetic solution whose composition closely paralleled that of the spinal fluid.

The present communication is concerned with the metabolism of tissue slices in cerebro-spinal fluid and in a synthetic fluid medium whose essential inorganic constituents equalled in concentrations those of spinal fluid.

The indirect Warburg[2] technique was used throughout, making it possible to measure the consumption of oxygen, Q_{O_2}, and the production of carbon dioxide, $Q^{O_2}_{CO_2}$. White rats of homogeneous strain were used in all experiments. The animals were killed by decapitation in a room maintained at 37°, the liver immediately sliced and placed in warmed salt solution. From this, they were transferred to their appropriate fluid medium and their metabolism measured at 38°. Observations were made at 15-minute intervals for a total of 75 minutes. The cerebro-spinal fluid used in the experiments was obtained through the kindness of the neurological services of the Boston City and Massachusetts General Hospitals, to

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[1] Davis, J. E., and Hastings, A. B., Proc. Soc. Exp. Biol. and Med., 1935, **32**, 1449.

[2] Warburg, O., *Biochem. Z.*, 1924, **152**, 51.

TABLE II.
Apparent Effect of Presence of Phosphate on Calculated Oxygen Consumption and CO_2 Production.

	PO_4 Absent		PO_4 Present (0.5 mM/L)	
	h	H	h	H
Manometer Reading, mm.	—8	—91	—20	—104
Oxygen Consumption, cmm.	127		125	
Carbon Dioxide Production, cmm.	129		110	

whom the authors are indebted. The fluid was drawn under sterile precautions and kept refrigerated until used. Immediately before use, it was passed through a Berkefeld filter.

In view of the impracticability of carrying out complete inorganic analyses of each sample of spinal fluid before use, average values of the inorganic ions of spinal fluid (except phosphate) were taken as the basis of the preparation of the synthetic medium. The composition of the synthetic medium is given in Table I. In all experiments, the concentration of inorganic phosphate was determined in each sample of spinal fluid, and a similar concentration was added to the synthetic medium with which each was compared. This was done in order to obviate the possibility of having differences in results which were due to differences in the buffer values of the spinal fluid and the synthetic medium.

A critical mathematical analysis of the differential method revealed that although the presence of 0.5 mM of inorganic phosphate per liter had a marked influence on the manometer readings, the calculated oxygen consumption was not appreciably altered. However,

TABLE III.
Oxygen Consumption and CO_2 Production of Liver in Spinal Fluid and the Synthetic Medium.

	Q_{O_2}			$Q^{O_2}_{CO_2}$		
	cmm./mg./hr.			cmm./mg./hr.		
Exp. No.	Spinal Fluid	Synthetic Fluid	Difference	Spinal Fluid	Synthetic Fluid	Difference
1	9.5	9.8	—0.3	11.1	11.6	—0.5
2	11.0	8.6	+2.4	12.8	10.0	+2.8
3	8.6	9.8	—1.2	7.3	9.6	—2.3
4	10.5	9.2	+1.3	11.5	12.0	—0.5
5	8.6	9.7	—1.1	8.5	11.2	—2.7
6	11.8	12.0	—0.2	9.0	8.5	+0.5
7	9.2	7.5	+1.7	10.3	9.5	+0.8
8	8.7	7.1	+1.6	10.0	7.9	+2.1
9	9.1	9.0	+0.1	10.6	10.6	0.0
Aver.	9.7	9.2	+0.5	10.1	10.1	0

the apparent carbon dioxide production is significantly reduced. An example of such a calculation is given in Table II.

Glucose was added to the spinal fluid to bring the concentration to approximately 0.2%. All experiments were run in duplicate. No specimens of spinal fluid were used which contained enough protein to give a visible precipitate with 20% trichloracetic acid.

The results of the comparison of the oxygen consumption, Q_{O_2}, and carbon dioxide production, $Q^{O_2}_{CO_2}$, of liver slices in 9 experiments are given in Table III.

It may be concluded from these experiments that the metabolism of rat liver in the synthetic medium herein used is not significantly different from that observed in cerebro-spinal fluid, when care is taken to make the composition of the synthetic medium approximately equal to that of the spinal fluid.

9535 P

Possible Rôle of Glutathione as a Detoxifying Agent.

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Aside from the probable rôle which glutathione plays in oxidative mechanisms, the suggestion has been advanced that it may be of importance as a detoxifying agent.[1] It is, to say the least, extremely suggestive that this polypeptide consists of 3 amino acids, each one of which is known to act as a detoxifying agent.

It occurred to us that experimental evidence for or against such a hypothesis might be gathered by injecting a substance which is known to form a detoxified product with one of the amino acids of the glutathione molecule, and then determining the glutathione content of the blood. We proceeded on the hypothesis that such an injection would be followed by a mobilization of the glutathione in order to furnish the required amino acid for detoxifying purposes.

Waelsch,[2] using tribromoethanol and phenylacetic acid, claims to have obtained a decrease in the glutathione of the blood.

The substances we selected for subcutaneous injection were benzoic acid and bromobenzene. The former is known to combine with glycocoll and the latter with cysteine; and both these amino acids are found in the glutathione molecule.

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[1] Harrow, B., and Sherwin, C. P., *Annual Rev. Biochem.*, 1935, **4**, 263.

[2] Waelsch, H., *Arch. Exp. Path. Pharm.*, 1933, **169**, 625.

Rabbits weighing about 2 kilos were used. Two gm. of benzoic acid (as the sodium salt) and an equivalent (molar) amount of bromobenzene were subcutaneously injected. The experiments were carried out with rabbits which had been fed, and also with rabbits which had been starved for 24 hours.

Blood was withdrawn for glutathione estimation, which was carried out according to Woodward and Fry.[3] The highest value within 3 hours after the injection was taken. We, of course, realize, as have others, that this method of glutathione determination has its shortcomings. For one thing, it is not specific for glutathione. For another thing, with small quantities of blood, extraordinary precautions have to be taken to avoid serious errors. However, we do believe that the figures can be taken as worthy of consideration in a *comparative* sense. [Our values for reduced glutathione for 34 normal fed rabbits ranged from 28 to 47 mg. %, with an average value of 33.0 mg. %. This agrees well with the findings of other workers (see, for example, Parker and Kracke[4]), who report an average value of 34.5 mg. % of reduced glutathione for a series of 24 normal rabbits.]

TABLE I.
Effect on Blood Glutathione (GSH) of Injected Benzoic Acid and Bromobenzene.

		No. Rabbits		Av. % Increase		Range	
		Control	Injected	Control	Injected	Control	Injected
Benzoic Acid (as sodium benzoate)	Fed	22	22	0.0	—0.6	—9.0 to 3.4	—8.5 to 10.5
	Starved	13	20	—0.6	20.9	—3.1 to 1.8	8.1 to 33.4
Bromobenzene	Fed	30	30	—0.3	3.8	—6.0 to 3.9	—9.9 to 14.4
	Starved	25	29	0.5	9.3	—12.0 to 5.4	3.3 to 23.0

The condensed results are represented in Table I. The figures are given in percentage increases (or decreases).

It can be stated that, in so far as animals which have been fed are concerned, the results are practically negative. But with the animals which had been starved, the results are suggestive, especially in the case of benzoic acid; for here it can be seen that there is a definite increase in the glutathione of the blood.

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[3] Woodward, G. E., and Fry, E. G., *J. Biol. Chem.*, 1932, **97**, 465.

[4] Parker, F. P., and Kracke, R. R., *Am. J. Clin. Path.*, 1936, **6**, 41.

Conclusions. 1. The injection of benzoic acid into rabbits which have been starved for 24 hours causes an increase of glutathione in the blood. 2. Using bromobenzene, the results, though less striking, are suggestive that this detoxication process is accompanied by a definite increase of glutathione in the blood.

9536

Hormones in Urine of a Normal Non-Pregnant Woman.*

ALLAN PALMER. (Introduced by C. D. Leake.)

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The gonadotropic and estrogenic hormone content of 24-hour urine specimens from normal women has been studied at weekly intervals.[1, 2] More recently the daily urinary excretion of hormones has been determined[3-8] with results which have demonstrated a point not shown in weekly assays, namely, that the gradual rises and falls in estrin excretion curves are sudden in their occurrence and are confined to certain days. If hourly examinations could be made perhaps still sharper peaks might be found. Cohen, *et al.*,[9] reported their observations pertaining to the combined and free state of estrin in the urine of pregnant women at the onset of labor. Is there sudden freeing and combining of estrogenic substances associated with events of the menstrual cycle? My report, developed from the study of the urine excreted by one woman during one menstrual cycle, suggests that ovulation and menstruation are possibly associated with changes in the chemical state of estrin. Gonad-

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* Supported by the Christine Breon Fund for Medical Research.

[1] Frank, R. T., *The Female Sex Hormone,* 1929, Charles C. Thomas, Springfield, Ill.

[2] Frank, R. T., and Salmon, U. J., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1237.

[3] Gustavson, R. G., and Green, D. F., *J. Biol. Chem.*, 1934, **105**, xxxiv.

[4] Smith, G. V. S., and Smith, O. W., *Am. J. Physiol.*, 1935, **112**, 340.

[5] Gustavson, R. G., Wood, T., and Hays, E., *J. Biol. Chem.*, 1936, **114**, xlvi.

[6] Smith, G. V. S., and Smith, O. W., *New England J. Med.*, 1936, **215**, 908.

[7] Gustavson, R. G., Hays, E. E., and Wood, T. R., *Proc. Am. Soc. Biol. Chemists*, 1937, **31**, xlii.

[8] Yerby, L. D., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 496.

[9] Cohen, S. L., McMaster, B. A., Marrian, G. F., and Watson, M., *Lancet*, 1935, **1**, 674.

otropic hormone studies in non-pregnant women have been less satisfactory due to the variation in methods and interpretations of results.

My method of estrogenic hormone extraction combines important features of several other methods.[4, 10–13] Each 24-hour urine specimen is made up to 1200 cc. with distilled water if it does not already exceed that volume. Of this, 700 cc. are used for estrin assay and 500 cc. for gonadotropic hormone assay. The 700 cc. portion is put directly into a continuous extraction apparatus and extracted with benzene for 24 hours. The collecting flask contains 100 cc. of a saturated sodium carbonate solution as a wash for the benzene extract containing the hormone. The latter is then transferred to a separatory funnel and washed with 100 cc. of 2½% HCl, saturated with NaCl, to remove any trace of alkali. Olive oil is added to the benzene solution, the benzene removed by steam heat and the final extract made up to 7 cc. with olive oil, thus making a 100-fold concentration of urine estrin in its free state. One hundred cc. of concentrated HCl are next added to the 700 cc. portion of urine and the mixture autoclaved for 1½ hours under 15 lb. pressure at 120°C. After autoclaving, the urine is reëxtracted with benzene for 24 hours and the extract washed and evaporated as before and put into olive oil as a 100-fold concentration of urine estrin in its combined state. Injections and assays are carried out on castrated mice and corrections for the 24-hour volume calculated exactly as described previously.[14] Values for less than 4.0 gamma of estrone per liter of urine are not determined in this series.

Gonadotropic hormone extraction is carried out on each 500 cc. portion of urine by sodium tungstate precipitation, following a procedure described by Freed and Hechter.[15] Here, also, an extract representing a 100-fold concentration of urine is prepared. This allows the use of 2 immature mice for each specimen, the equivalent of at least 200 cc. of urine being injected into each test animal.

There is a difference of opinion in the interpretation of findings in gonadotropic hormone assay of the urine. All agree that production of corpora lutea in the ovaries of immature normal mice or rats after 100 hours indicates the presence of luteinizing hormone, the

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[10] Kurzrok, R., and Ratner, S., *Am. J. Obst. and Gynec.*, 1932, **23**, 689.

[11] Schmulovitz, M. J., and Wylie, H. B., *J. Lab. and Clin. Med.*, 1935, **21**, 210.

[12] Cohen, S. L., and Marrian, G. F., *Biochem. J.*, 1935, **29**, 1577.

[13] Schmulovitz, M. J., and Wylie, H. B., Mimeographed Supplement to Reference No. 11.

[14] Palmer, A., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 123.

[15] Freed, S. C., and Hechter, O., *Endocrinology*, 1936, **20**, 396.

amount being calculated according to the quantity of urine injected. This reaction denotes the presence of the gonadotropic hormone characteristic of pregnancy. There is also agreement that in a similar manner the production of many uniformly large follicles, without hypertrophy of the interstitial or theca cells in the ovaries of immature hypophysectomized mice or rats after 100 hours, indicates the presence of follicle stimulating hormone in the preparation injected and that this reaction denotes the presence of the urinary gonadotropic hormone characteristic of the menopause.

CHART

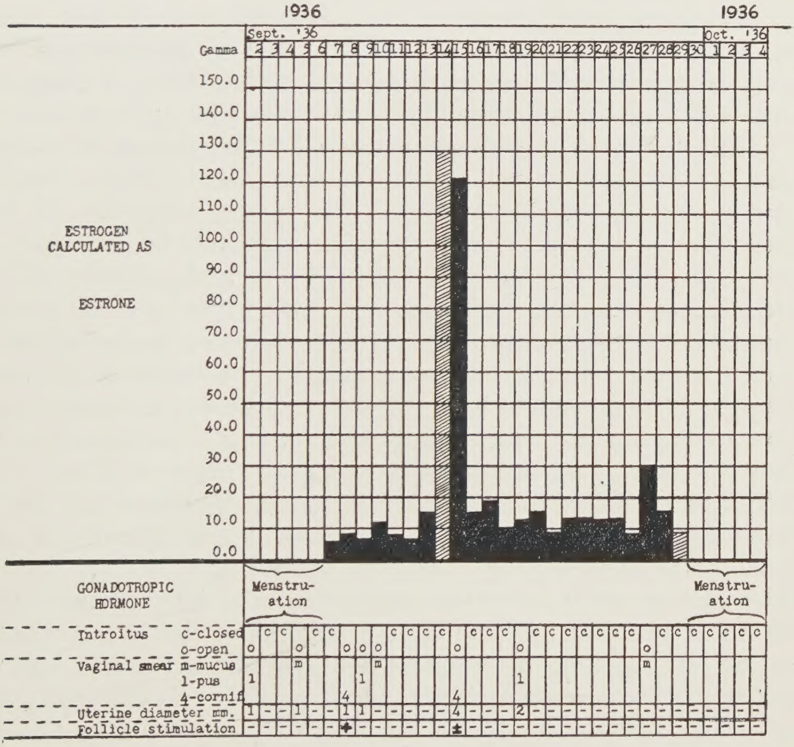

FIG. 1.

The chart shows graphically the excretion of estrogenic hormone. The solid columns represent combined estrin and the shaded columns free estrin both of which values are calculated as estrone. The lower section of the chart lists the findings in gonadotropic hormone detection.

Others believe that such criteria as opening of the vagina, increase in uterine and ovarian size and weight, histologic evidence of some follicle development and theca and interstitial cell hypertrophy in immature normal female mice or rats are reactions on which may be based the assay of gonadotropic hormone in the urine when neither pregnancy nor the menopause is concerned. The finding of uterine enlargement in immature test animals by some workers has been their basis for reporting the presence of gonadotropic hormone even though follicle growth has been absent.[16] My gonadotropic hormone extracts, tested on normal immature female mice, were effective in producing the above named criteria in a few instances during the one menstrual cycle studied. An interstitial cell stimulating hormone of the pituitary in the urine apparently is not demonstrable when immature normal rodents are used.[17] Follicle growth, as characterized by many thin-walled, uniformly enlarged follicles without an associated hypertrophy of interstitial and theca cells was not seen. Corpora lutea were never found. In brief, neither the gonadotropic hormone characteristic of pregnancy nor that characteristic of the menopause was found at any time during the menstrual cycle studied.

Estrin equivalent to amounts greater than 4.0 gamma of estrone per liter of urine was not excreted in the urine in either chemical form during menstrual bleeding. The sudden excretion of free estrin for one day (9-14-36) might be explained on the basis of sudden peritoneal absorption of follicular fluid and excretion of free estrin before adequate detoxication or combination with glucuronic acid could occur, which process may have occurred by the following day (9-15-36). The second occurrence of free estrin in the urine is hardly great enough to be significant. However, a comparison is made of this finding with those of Cohen, *et al.*,[9] pertaining to the chemical state of estrogenic hormones at the onset of labor. The latter and the onset of menstruation can be considered as similar physiologic processes. In both instances uterine activity is preceded by the occurrence of free estrin in the urine. At the onset of labor the best outlet for the estrogenic hormone is by kidney excretion which probably accounts for the remarkable rise in free urinary estrin. At the time of menstruation free urinary estrin has occurred, but the onset of bleeding, which possibly effects a ready outlet for the hormone, might account for the disappearance of the hormone from the urine during the menstrual period. The high estrin content of menstrual blood has been demonstrated.[1, 18]

Summary. This report concerns the daily excretion of estrogenic hormone in both free and combined chemical forms during one menstrual cycle of a normal multiparous woman. The possible excretion of gonadotropic hormone is reported merely that comparisons may be made with the reports of other workers. I feel that the excretion of this has not been demonstrated. Evidence of a significant association between the events of the menstrual cycle and changes in the amount and chemical form of estrin is discussed.

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[17] Evans, H. M., Korpi, K., Simpson, M. E., Pencharz, R. I., and Wonder, D. H., *Univ. California Publ. Anat.*, 1936, **1**, 255.

[18] Frank, R. T., and Goldberger, M. A., *J. A. M. A.*, 1926, **86**, 1686.